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CHROMOSOME NUMBERS OF THREE MOUSE ASCITES TUMOURS

BY *ALBERT LEVAN* AND *THEODORE S. HAUSCHKA*

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ALTHOUGH several papers on the Ehrlich ascites tumour of the mouse have touched upon the chromosome conditions of this tumour, practically no exact chromosome number counts have so far been published. KLEIN (1951, and elsewhere) has determined the DNA content of various ascites tumours. He found the very interesting situation that different tumours behaved differently in this respect; the three studied lymphomas had about the same content of DNA as normal diploid cells of resting tissues in the mouse, whereas the studied carcinomas and sarcomas had about double the content of DNA.

HAUSCHKA and LEVAN (1951) observed a striking correlation between KLEIN's DNA determinations and the chromosome numbers actually counted in the same tumours. On that occasion only a very short abstract was published, which is now supplemented with data from chromosome number determinations in 50 or 100 cells in three ascites tumours, *viz.*, the DBA lymphoma, the Ehrlich, and the Krebs 2 carcinomas.

The study was made on cells simultaneously fixed and stained in aceto-orceine, and thereafter pressed out between siliconed surfaces of the slide and the coverslip (SCHULTZ' method, unpublished). The quality of the slides may be seen in Fig. 1. It is also seen from this picture that chromosomes of different tumours show striking differences in the readiness with which they can be analysed. While the large-celled Ehrlich and Krebs 2 are extremely favourable for chromosome study, the DBA lymphoma is decidedly less favourable. Its smaller cells do not allow the chromosomes to spread out as freely as they do in the former tumours. In the DBA lymphoma 50 metaphase plates were analysed, the error being $\pm$ 0—2 chromosomes. In Ehrlich 50 plates and in Krebs 2 100 plates were counted. Most of these counts were exact, but for the higher numbers an error of $\pm$ 1 or, in rare cases, 2 chromosomes may occur.

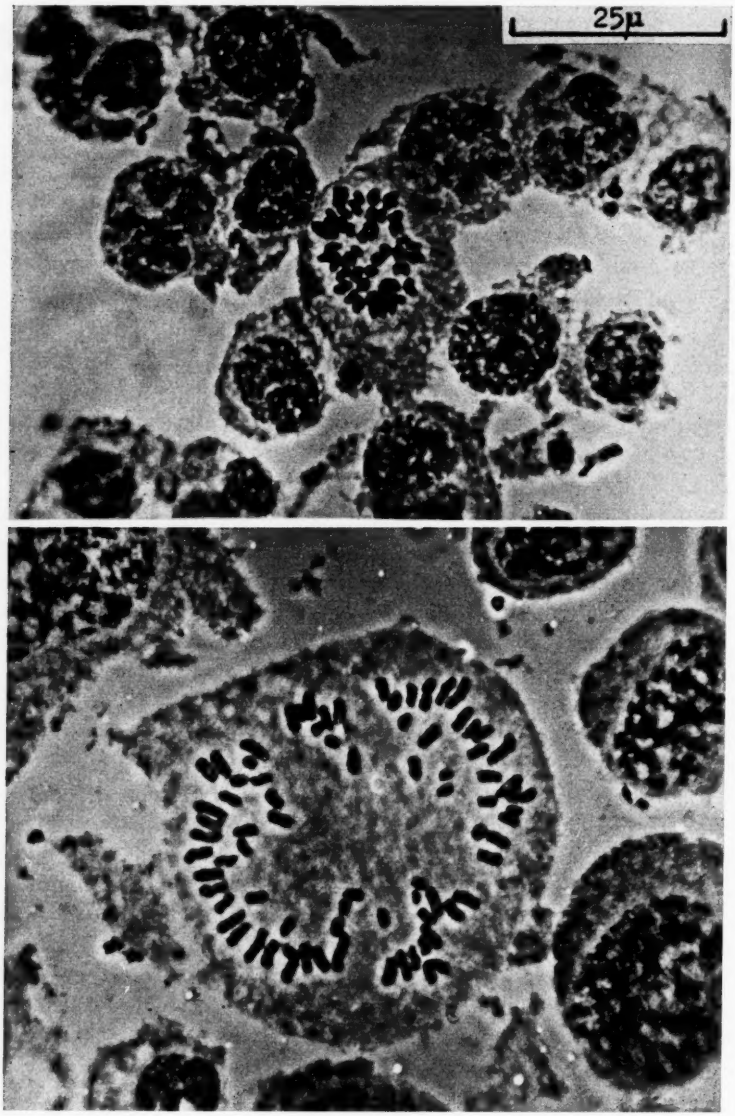

Fig. 1. Metaphase plates of *a*: DBA lymphoma, and *b*: Krebs 2 carcinoma. Microphotographs in phase contrast.

TABLE 1. *Distribution of chromosome numbers over the whole range.*

Tumour	Middle chromosome number of each class																				Total
	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	— 145 —	160	165	170	
DBA lymphoma	1	29	15	2	—	1	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	50
Ehrlich	—	1	—	—	—	—	1	1	6	27	10	2	—	1	—	—	—	1	—	—	—	50
Krebs 2	1	3	2	2	1	2	3	3	18	35	15	1	2	2	1	2	1	1	2	1	2	100

TABLE 2. *Constitution of the modes of Table 1.*

Mode at diploid number	Chromosome number																Total
	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50		
DBA lymphoma	1	—	—	1	5	9	14	4	4	7	—	—	—	—	2		47
Mode at tetraploid number	**Chromosome number**																
	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	
Ehrlich	1	1	—	1	2	2	2	4	9	4	8	5	1	2	2	—	44
Krebs 2	1	3	1	3	4	7	6	4	9	8	8	3	4	5	—	3	69
Total 4x tumours	2	4	1	4	6	9	8	8	18	12	16	8	5	7	2	3	113

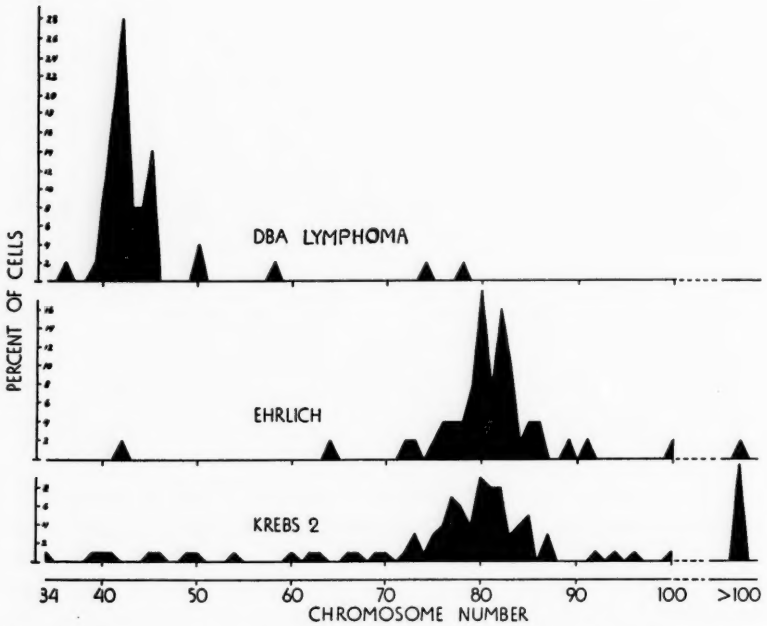

Fig. 2. Graph showing the distribution of chromosome numbers in the three ascites tumours studied.

The chromosome numbers recorded are collected in Tables 1 and 2, and in the graph of Fig. 2. Table 1 gives the numbers of the entire range collected into classes of 5 numbers. The difference in chromosome number distribution between the DBA lymphoma, on the one hand, and the Ehrlich and Krebs 2 carcinomas, on the other, is quite striking. Whereas most of the DBA lymphoma cells have numbers in the vicinity of the diploid number of the mouse ($2n=40$), the two carcinomas have a much wider range of chromosome numbers, most of them being gathered around the tetraploid number ($2n=4x=80$). The detailed constitution of the mode of each tumour is seen in Table 2. The DBA lymphoma has a rather narrow mode with its mean somewhat above the diploid number, 42 being the number most commonly represented. The modes of the tetraploid tumours are more flattened, the classes 80—82 being most frequent. It is of interest that the DBA lymphoma has occasional cells in the tetraploid region (two cells in the material recorded), whereas Ehrlich and Krebs 2 have clear indications

of a second mode at and below the octoploid number. While no cells of Ehrlich and Krebs 2 were found with numbers between 114 and 142, seven cells had numbers between 143 and 171. Both Ehrlich and, above all, Krebs 2 have cells going down below the modal region at $4x$, occasionally even subdiploid cells being encountered. In Krebs 2 there is some tendency to a third mode at the diploid region of the distribution curve, eight cells having numbers between 34 and 50.

It is evident from the above data that among the mouse ascites tumours there occur such as are characterized by a diploid modality of chromosome number, while in other tumours most of the cells have approximately tetraploid nuclei. Each tumour may be looked upon, from a cyto-genetical point of view, as a cell population of varying chromosome numbers. Since each tumour maintains its characteristic type throughout many transplant generations, it is evident that the relative viability of the various chromosome numbers keeps the population at an equilibrium. In other words, each tumour is propagated by a certain »germ-line», while the frequent deviations in chromosome number from this germ-line represent more or less unsuccessful chromosome combinations, which ordinarily are of no consequence for the continuity of the tumour. The germ-line may be constituted by cells with $\pm$diploid chromosome number, such being the case with the DBA and the 6C3HED lymphomas. It may, in other cases, consist of cells close to the tetraploid number, as in Ehrlich, Krebs 2 carcinomas and MC1A rhabdomyosarcoma (HAUSCHKA and LEVAN, 1951). Each tumour is thus characterized cyto-genetically by its population of chromosome numbers, which of course is an expression of the types and frequencies of mitotic disturbances in the tumour, as well as the relative viability of the different chromosome combinations originating from these disturbances.

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CYTOLOGICAL STUDIES IN EAST AFRICAN MOUNTAIN GRASSES

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INTRODUCTION.

DURING the Swedish East Africa Expedition of 1948, the present author studied the alpine flora and vegetation of the high mountains of East Africa (cp. HEDBERG, 1951). One of his main objects was to investigate species differentiation within the »afro-alpine» flora. For that purpose attempts were made to procure cytological material of as many alpine plants as possible, in order to utilize cyto-taxonomical data for the taxonomical classification. Lack of time and equipment prevented more detailed cytological studies, and in the first place an endeavour was made to establish the chromosome numbers of the alpine plants. In this paper are included the results obtained for the *Gramineae*. The taxonomical treatment of the grasses collected is being made by Dr. A. MELDERIS, and the aim of the present communication is to provide some cytological basis for that treatment.

METHODS.

All fixations were made in chrome-acetic formalin (MÜNTZING's modification of NAVASHIN's fluid; cp. MÜNTZING, 1933, p. 131). The material was later embedded in paraffin, sectioned with microtome, stained with crystal violet, and mounted in Canada balsam. The material for fixation, consisting of root-tips and buds, was as a rule collected from the same specimens that were afterwards put into press. Thus each fixation made corresponds to one herbarium number, usually comprising 4 sheets, which will be distributed to public herbaria (cp. HEDBERG, 1951, p. 142). The fixation was generally made in the evening or at night, when the day's harvest was being prepared. It might be surmised that with this method the fixations would be bad, since they were usually not made until 10—12 hours after the plants were collected. The plants were kept in a moist atmosphere in water-tight bags, however, and the results were mostly satisfactory.

The diameters of pollen grains quoted in a couple of cases below were measured on material from herbarium specimens, partly from my own collections, and partly from the Botanical Museum, Uppsala. Dry stamens were transferred to a drop of lactophenol on a microscopic slide; and the preparation was heated gently, sealed, and left over night. The measurements were made the following day. In each sample 20 grains were measured, if not otherwise stated.

Owing to the vicissitudes of the home-transport, some of my fixations dried. As the material in question could not be replaced, it was attempted to find a method to save it.

VAARAMA (1950) describes a method for dry-preservation of fixed plant material. The material is transferred from the fixative to 70 % alcohol (3 changes) and desiccated. When required for study, it is swollen by being kept for 2 days in 10 % alcohol and then embedded in paraffin in the usual way.

In order to test whether the same method could be applied when the fixed material had been desiccated in the fixative, a small experiment was carried out. Three series of fixations were made in chrome-acetic formalin, each comprising 4 fixations of different plants (viz. *Lobelia Burttii* E. A. BRUCE, *Zea mays* L., *Polygonum alatum* BUCH.-HAM., and *Senecio vulgaris* L.). The first two series were desiccated and left dry for 2 months, whereas the third was kept as control. The first series was then treated with 4 % formaldehyde (commercial formalin diluted 1 : 10) for 1 week, and the second with 10 % alcohol for 4 days. Finally, all 3 series were embedded in paraffin and sectioned, stained with crystal violet, and mounted in Canada balsam. Satisfactory results were obtained for the first 3 plants in all series, whereas the *Senecio* fixation gave poor results in all 3 series. No significant difference was found with the two methods used for swelling.

After this preliminary test, all my dried field fixations, which had been dry for more than 2 years, were swollen with 10 % alcohol for one week and then treated in the same way as above. The results were quite satisfactory: out of 14 dry fixations 10 could be counted. Good results were obtained with plants from the following genera: *Agrostis*, *Deschampsia*, *Luzula*, *Myosotis*, *Pentaschistis*, *Poa*, *Ranunculus*, *Subularia*, and *Swertia* (cp. also above).

The above results show that in most cases fixations in chrome-acetic formalin, that have dried, may be rescued by swelling for a few days in 10 % alcohol or 4 % formaldehyde before embedding in paraffin. The material will usually be rather brittle, and the sections are

sometimes apt to fall off the slides in making the preparations, but the material may well be utilized. The same method may probably be also used for rescuing dried fixations of other types (cp. VAARAMA, 1950).

SURVEY OF CHROMOSOME NUMBERS IN THE GENERA INVESTIGATED.

For definite names of the plants the reader is referred to a coming publication by Dr. A. MELDERIS. The numbers quoted for the specimens are my field numbers.

AGROSTIS.

In the alpine and subalpine species of this genus the following chromosome numbers have been found:

$2n=28$: Hedb. 412 (Ruwenzori); 1543 and 1664 (Aberdare); 1724, 1725, 1768 and 1907 (Mt. Kenya); 2113, 2247 and 2249 (Muhavura); 2331 (Mt. Meru).

$2n=42$: Hedb. 1248 (Kilimanjaro).

The name *Agrostis chionogeiton* was given by PILGER (in FRIES und FRIES, 1926, p. 510) to a new grass from Mt. Kenya. The same grass was collected by me in the alpine belt on Mt. Kenya (Hedb. 1759, 1902; observed only at an altitude of 4200—4400 m) and Kilimanjaro (Hedb. 1253; only found at an altitude of 4300—4700 m). When the material was examined by Dr. A. MELDERIS, he found it to represent a new genus, not closely related to *Agrostis*. This was strongly supported by the chromosome number, which was found to be $2n=8$ on all three collections mentioned. The same number was obtained for an undescribed grass from Mt. Elgon (Hedb. 908; observed at altitudes of 3600—4000 m). On morphological grounds Dr. MELDERIS independently found this species to belong to the same new genus.

The chromosomes of these two species are of about equal length, all with submedian centromere (cp. Figs. 1—4). Two of them possess marked secondary constrictions, which with the fixative used were best discovered at anaphase (cp. Fig. 1), but were also discernible in part of the metaphase plates.

The basic number 5 was considered by FLOVIK (1938) to be the most primitive in the *Gramineae* (cp. also LITARDIÈRE, 1948). The basic number 4 has earlier only been found in two grasses, *Airopsis tenella* (CAV.) COSS. et DR., and *Periballia laevis* (BROT.) ASCH. et GRAEBN.

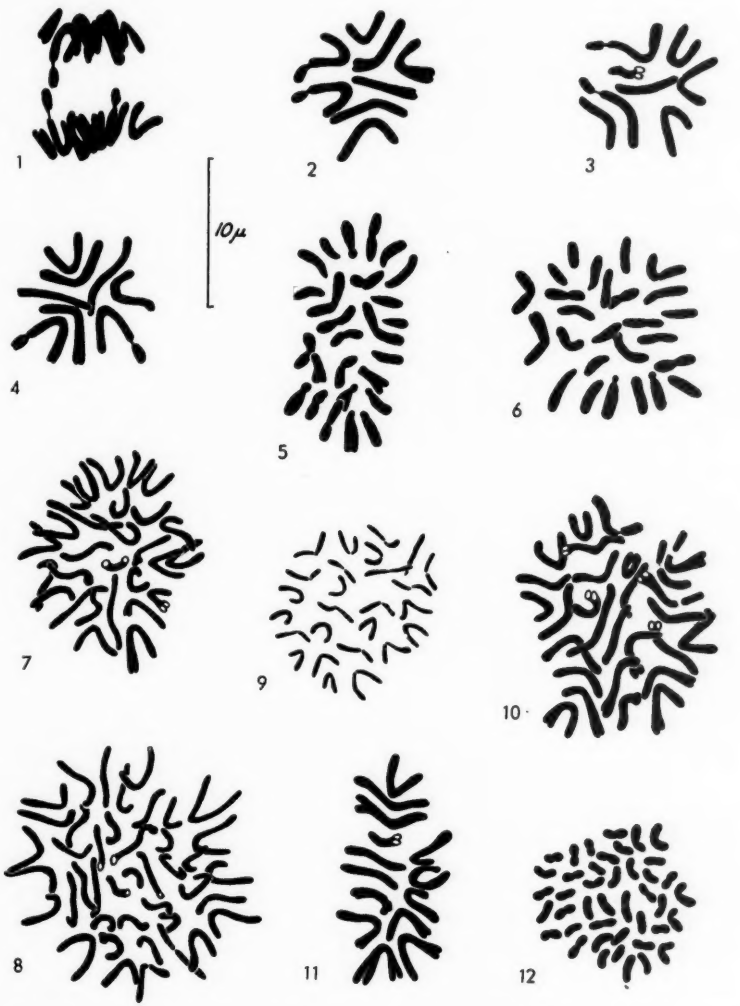
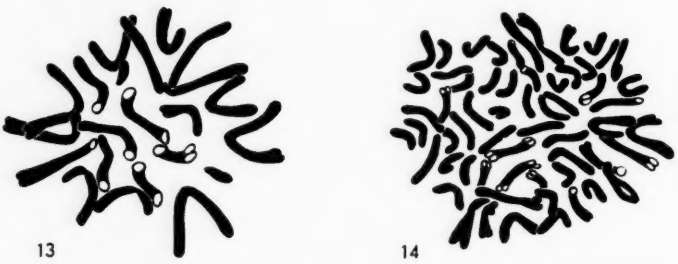


Fig. 1, anaphase; Figs. 2—12, metaphases, all from somatic divisions in root-tips. — 1—2, Hedb. 908, $2n=8$. — 3, »*Agrostis chionogeiton*», Hedb. 1253, $2n=8$. — 4, Do., Hedb. 1759, $2n=8$. — 5, *Deschampsia* sp. near *caespitosa*, Hedb. 363, $2n=26$. — 6, Do., Hedb. 860, $2n=26$. — 7, *Deschampsia flexuosa*, Hedb. 1361, $2n=28$. — 8, Do., Hedb. 389, $2n=42$. — 9, *Pentaschistis* sp., Hedb. 1176, $2n=26$. — 10, *Festuca* sp., Hedb. 1901, $2n=28$. — 11, *Koeleria* sp., Hedb. 1873, $2n=14$. — 12, *Poa* sp., Hedb. 402, $2n=42$.

(LITARDIÈRE, *op. c.*). The latter number has probably been derived from the former, as suggested by LITARDIÈRE. In any case, the 8-chromosomic grasses certainly merit further cyto-taxonomic study.

ANTHOXANTHUM.

This genus is known to have the basic number 5; earlier the somatic numbers 10, 20, and 80 have been recorded (ÖSTERGREN, 1942). The only species so far reported from the East African mountains is *A. nivale* K. SCHUM., occurring in the alpine and ericaceous belts. Three of the samples investigated cytologically (Hedb. 1537 from Aberdare,



13 14

Figs. 13—14, metaphases, from somatic divisions in root-tips. — 13, *Anthoxanthum* sp., Hedb. 2183, $2n=20+2$ f. — 14, *Anthoxanthum* sp., Hedb. 1723, $2n=60$.

1723 and 1779 from Mt. Kenya) proved to have $2n=60$, *i. e.* the dodecaploid number (cp. Fig. 14). Slight irregularities occur, as is natural with such a high degree of polyploidy, thus in one root tip of the sample Hedb. 1723 $2n=$c. 64 was counted (cp. ÖSTERGREN, *op. c.*).

One sample of *Anthoxanthum* from Muhavura (Hedb. 2183) proved, however, to have instead the tetraploid number, 20, + 2 fragments (cp. Fig. 13). The fragments probably correspond to the B-chromosomes described in *Anthoxanthum aristatum* by ÖSTERGREN (1947). When the pollen size was measured of the cytologically investigated specimens as well as other herbarium material of »*Anthoxanthum nivale*» available at Uppsala, it was found that the pollen grains of the known 20-chromosomic type were smaller than those of all other samples; the difference was found to be statistically significant ($P < 0{,}001$); cp. Tables 1 and 2.

TABLE 1. *Pollen diameter in Anthoxanthum.*

Origin of sample	Pollen diameter (μ)	Chromosome number (when known)
Uganda, Mt. Muhavura, Hedb. 2183 (100 grains measured)	37,3 ± 0,23	20+2f
» , Ruwenzori, Hedb. 351	41,0 ± 0,46	—
Tanganyika Territory, Kilimanjaro, Hedb. 1200	46,7 ± 0,58	—
» » , Mt. Meru, Hedb. 2304	40,1 ± 0,35	—
Kenya Colony, Mt. Aberdare, Hedb. 1537	46,4 ± 0,46	c. 60
» » , » » , Fries and Fries 2916	45,9 ± 0,36	—
» » , » » , » » » 2474	43,2 ± 0,42	—
» » , » » , » » » 2361	41,9 ± 0,50	—
» » , Mt. Kenya, Hedb. 1723	49,0 ± 0,29	c. 60—64
» » , » » , » 1779	43,6 ± 0,20	c. 60
» » , » » , Fries and Fries 1287	46,6 ± 0,46	—
» » , » » , Do, another sheet	42,3 ± 0,58	—
» » , » » , Fries and Fries 1287 a	43,6 ± 0,46	—
» » , » » , » » » 1381	44,7 ± 0,48	—

TABLE 2. *Analysis of variance of pollen size in Anthoxanthum.*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Between samples	5094,68	13	391
Between Hedb. 2183 and the other samples	3486,2	1	3486
» the other samples	1608,48	12	134
Within samples	1483,54	346	4,29
Total	6578,22	359	

Ratios:

$$\frac{391}{4{,}29} = 91{,}4;\ P < 0{,}001$$

$$\frac{3486}{134} = 26{,}0;\ P < 0{,}001$$

DESCHAMPSIA.

(a) *Deschampsia sp.* [near *caespitosa* (L.) PB.]. The chromosome number of Scandinavian *D. caespitosa* appears to be $2n=26$, and in non-sexual strains of the closely related *D. alpina* (L.) R. et S. other multiples of 13 have been found (cp. LÖVE and LÖVE, 1948; TISCHLER, 1950). The types occurring on the East African mountains (mainly in the ericaceous belt) are certainly closely related to *D. caespitosa* and the somatic chromosome number could also be safely determined to 26 on two samples (Hedb. 363 from Ruwenzori, Hedb. 860 from Mt. Elgon; cp. Figs. 5 and 6).

(b) *Deschampsia flexuosa* (L.) TRIN., coll., has a very wide distribution, occurring also on the mountains of East Africa, mainly in the ericaceous belt. In this species only the somatic chromosome number 28 (tetraploid) seems to have been reported earlier (cp. LÖVE and LÖVE, *op. c.*, TISCHLER, *op. c.*). Three of my collections also gave that number (Hedb. 1361 from Kilimanjaro, cp. Fig. 7; Hedb. 1840 from Mt. Kenya, and Hedb. 2075 from Muhavura), whereas one collection from Ruwenzori (Hedb. 389, Fig. 8) had $2n=42$ (hexaploid). For comparison the

TABLE 3. *Pollen diameter in Deschampsia flexuosa.*

Origin of sample	Pollen diameter (μ)	Chromosome number (when known)
Uganda, Ruwenzori, Hedb. 389 (100 grains measured) ...	38,1 ± 0,26	42
» , Mt. Muhavura, Hedb. 2075	33,9 ± 0,46	28
Kenya Colony, Mt. Elgon, Hedb. 931	26,8 ± 0,23	—
» » , » Kenya, » 1840	28,5 ± 0,39	28
» » , » » , » 1986	32,1 ± 0,48	—
» » , » » , Fries and Fries 1286	34,7 ± 0,28	—
» » , » Aberdare, Fries and Fries 2404	31,5 ± 0,37	—
Tanganyika Territory, Kilimanjaro, Hedb. 1361	30,9 ± 0,35	28
» » , Mt. Meru, Hedb. 2394	30,0 ± 0,30	—
Sweden, Skåne, Ö. Hoby, 3.7.1944, N. Johansson	31,5 ± 0,24	—
» , Uppland, Frötuna, 1875 (ex herb. De Geer)	30,1 ± 0,33	—
» , » , Värmdö, 1906, H. Smith	28,4 ± 0,25	—
» , » , Uppsala, 1877, G. Moll	29,4 ± 0,34	—
» , » , V. Löfsta, 1881, C. A. E. Lenström	30,6 ± 0,34	—
» , » , Torsvi, 1882, K. J. Hæggblom	31,1 ± 0,48	—
» , Medelpad, Häfverö, 1880, K. F. Dusén	31,4 ± 0,28	—
» , Jämtland, Tännforsen, 1911, H. F. Lundberg	30,5 ± 0,38	—
» , » , Åreskutan, 1891, I. Collijn	31,2 ± 0,11	—
» , Torne Lpm., Kiruna, 1905, K. Johansson	30,3 ± 0,45	—
» , » » , Jebrenjokk, 1913, L. Trafvenfelt	31,7 ± 0,40	—
» , » » , Nakerijokk, 1905, K. P. Hägerström	29,9 ± 0,35	—
Norway, Vestfinnmark, Sorö, 1880, C. Lindman	30,5 ± 0,34	—
Germany, Lübeck, 1901, G. Kjellberg	28,1 ± 0,20	—
Scotland, Argyll, 1949, A. Gaudson	28,7 ± 0,30	—
France, »The Pyrenees», 13.8.1856, J. E. Zetterstedt	32,5 ± 0,40	—
Greenland, J. Vahl	30,2 ± 0,33	—
» , Godhavn, G. Arik	31,9 ± 0,46	—
Canada, Nova Scotia, Howe and Lang 665	28,0 ± 0,35	—
» , Quebec, J. A. Calder 2432	29,3 ± 0,30	—
U. S. A., New York, Mt. Marey, 1839, W. F. Maerae	29,5 ± 0,43	—
S. America, Tierra del Fuego, 1896, P. Dusén	30,1 ± 0,32	—
Falkland Islands, 2.2.1908, C. Skottsberg	28,6 ± 0,34	—
Mean value for the African samples except Hedb. 389	31,03 ± 0,233	—

TABLE 4. *Analysis of variance of pollen size in Deschampsia flexuosa.*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Between samples	6928,84	31	224
Between Hedb. 389 and the other samples	5143,67	1	5144
» all remaining samples	1785,17	30	59,5
Between remaining African samples	971,30	7	139
» all non-African samples	718,87	22	32,7
» African—non-African samples	95,00	1	95
Within samples	2172,09	688	3,16
Total	9100,93	719	

Ratios:

$$\frac{224}{3,16} = 70,9;\ P < 0,001$$

$$\frac{5144}{59,5} = 86,5;\ P < 0,001$$

$$\frac{139}{32,7} = 4,25;\ 0,001 < P < 0,01$$

pollen diameter was measured on these collections as well as on a number of other ones from different localities (Table 3). Analysis of variance (Table 4) shows the variance between samples to be significantly larger than within samples ($P < 0,001$). There is also a significant difference between the first sample of Table 3 (Hedb. 389) and all the others ($P < 0,001$). The values of Table 4 further suggest a higher degree of variation between the East African samples than between samples from other continents, though here the evidence is less satisfactory ($0,001 < P < 0,01$). These results indicate, in the first place, that the hexaploid has larger pollen grains than the tetraploid, and, secondly (as may also be inferred from the chromosome determinations so far made), that the hexaploid must be rather uncommon. As no close relative of *Deschampsia flexuosa* (s. lat.) occurs on the East African mountains — *D. ruwenzoriensis* CHIOV. (CHIOVENDA e CORTESI, 1909, p. 468) is hardly distinct from *D. flexuosa*; cp. PILGER in FRIES und FRIES, 1926, p. 516 —, and, further, the morphological differences between the two types seem to be very slight, the hexaploid type on Ruwenzori has probably originated as an autopolyploid by fusion of an unreduced egg-cell with a reduced male nucleus.

The higher variance of pollen size between East African samples as compared with samples from other parts of the world may possibly indicate a higher degree of diversification of the species here than

elsewhere, promoted by its occurrence in a number of relatively small and strictly isolated mountain populations. It should be noted that considerable differences in pollen diameter were also found between different samples from the same mountain (Mt. Kenya; cp. Table 3).

The ratio between the squares of the mean diameter for the pollen grains of sample Hedb. 389 on one hand and all the other African samples on the other hand equals 1452: 963 or 1,51 : 1, which is in good agreement with the hypothesis that nuclear (and cell) surface is proportional to chromosome number in closely related forms (cp. GEITLER, 1938, p. 125).

FESTUCA.

The somatic chromosome number was determined to 28 on the samples Hedb. 1862, 1901 and 1928, all from Mt. Kenya. In sample Hedb. 1901 four chromosomes with distinct secondary constrictions could be discerned (cp. Fig. 10).

KOELERIA.

The somatic chromosome number 14 was found in material from Mt. Kenya (Hedb. 1873); cp Fig. 11.

PENTASCHISTIS.

The following somatic chromosome numbers were found:

$2n=26$: Hedb. 1176 (Kilimanjaro)

$2n=52$: Hedb. 1773 (Mt. Kenya); 2329 (Mt. Meru).

A fourth collection of *Pentaschistis* (Hedb. 1538 from Mt. Aberdare) gave $2n=$ c. 39—40 (no exact count was possible because of bad fixation). The basic number is obviously 13, hence a triploid hybrid would of course have $2n=3x=39$. The hybrid nature of these specimens was supported by an investigation of the pollen. Out of 3 specimens in flower one had normal, plasma-filled pollen grains (aver. diam. $36{,}3 \pm 0{,}34\ \mu$), whereas 2 had poor pollen with irregular, empty grains (aver. diam. $25{,}3 \pm 1{,}80\ \mu$), among which appeared, however, some few (less than 1 %) grains of normal size ($35{,}1 \pm 1{,}12\ \mu$) and containing plasma. The latter probably contained restitution nuclei.

Apparently this collection (Hedb. 1538) contains a triploid hybrid, from which root-tips were taken for fixation, together with one of its parents. No good correlation was found between chromosome number and pollen size in this genus (pollen diam. in the diploid Hedb. 1176 $=31{,}8 \pm 0{,}44\ \mu$, in the tetraploid Hedb. 1773 $=33{,}5 \pm 0{,}40\ \mu$).

The genus *Pentaschistis* has its main distribution in South Africa, being represented also, *i. a.*, on the high mountains of Tropical Africa. It was established by STAPF (1899), having earlier been treated as a subgenus of *Danthonia*. Cytological data seem to support the independence of the genus, since the East African species investigated have the basic chromosome number 13, whereas the basic numbers reported from *Danthonia* s.str. are 7, 9, and 12 (DARLINGTON and JANAKI AMMAL, 1945).

POA.

This genus occurs in a number of species on the high mountains of Tropical Africa. Most of them seem to have the somatic chromosome number 42 [counted in Hedb. 402 (cp. Fig. 12), 404, 499, 601, 602, 677, and 717 from Ruwenzori; 886 and 983 from Mt. Elgon; 1246, 1247, and 1262 from Kilimanjaro; 1741, 1757, 1780, 1781, 1956, and 1965 from Mt. Kenya; 2064, 2079, 2145, and 2188 from Muhavura; and 2342 and 2406 from Mt. Meru]. One single collection gave $2n$=c. 28 (Hedb. 2020 from Mt. Kenya).

In this part of the world all species appear to be normally sexual in contrast to the conditions in Arctic and Boreal countries (cp. NYGREN, 1950), and no case of vivipary has been recorded.

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STUDIES ON X-RAY INDUCED MUTATIONS IN LUPINUS LUTEUS L.

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I. INTRODUCTION.

BREEDING work with sweet lupines was started in Sweden in the middle of the 1930-ies, the development of earliness being one of the major breeding problems. No success was obtained in the yellow sweet lupines with the orthodox methods, and since in other plants it had been demonstrated beyond any doubt that earliness might be obtained by X-ray induced mutations without severe losses in vigour, work with such mutations was started in 1942.

The main purpose of the work was to obtain agriculturally valuable characters. At the same time, however, several phenomena occurring in the material were studied from a more theoretical point of view, and the results of these theoretical studies are reported here.

The yellow lupine is not a favourable object for theoretical genetical studies. The individual plant occupies considerable space, thereby increasing the experimental costs. Artificial crosses are difficult to make and result in a very low percentage of seed setting. Several other technical difficulties are met with, among which the most important one is the high degree of vicinism, about 25 % under the conditions prevailing in our experiments (HAGBERG, 1952). Any accurate genetical analysis would require artificial isolation of a large number of plants, a labour that has been outside our resources in time and money. Finally, *Lupinus luteus* has a chromosome number of $2n=52$, and the chromosomes are rather small. It is often difficult even to determine exactly the number present in a metaphase plate, and no technique, known to us, makes it possible to study in detail the morphology of the chromosomes or their behaviour during meiosis.

The technical difficulties and the impossibility of correlating any results obtained in the macroscopic analysis with exact cytological observations are the reasons why we have decided to discontinue the theoretical studies, with the possible exception of some phenomena which may give clear-cut experimental results even in this material.

The data here presented are, in many respects, rather fragmentary and the lack of »finish» is sometimes irritating. It is hoped, however, that the material, such as it is, by providing data from a plant otherwise scantily studied in regard to its reaction to X-rays, may be of some value in widening the general knowledge about the effect of mutagents on different organisms.

All during the work we have been in close contact with Professor Å. GUSTAFSSON, the leading spirit in Swedish investigations on induced mutations, and we wish to express our thanks for his interest and for valuable advice and suggestions. The work has partly been made possible by subsidies from the Knut and Alice Wallenberg fund, granted to Professor GUSTAFSSON and distributed by him for different investigations concerning induced mutations.

II. MATERIAL AND METHODS.

Throughout the experiments here recorded the seed treated by X-rays has been derived from different substrains within the original German strain 8 (V. SENGBUSCH, 1942) of sweet yellow lupine; except in the few cases where bitter yellow or even blue lupine has been used. In the years 1942—1950 the X-ray treatment of the seeds was made at the Radiophysics Institute in Stockholm (see MAC KEY, 1951), from 1951 on the new equipment of the Institute of Genetics at the University of Lund has been used. The seeds treated as well as the controls have always been of last year's harvest and they have been stored and handled in the laboratories of the Swedish Seed Association; no attempts have been made to standardize the conditions in regard to water content and other factors.

The seeds have been sown individually spaced in rows 50 or 45 cm apart with 10 cm between the plants in a row. In X-2 and later generations usually only one row — 14 or 15 seeds — has been sown with the progeny of any one plant in the preceding generation. The effects of this limitation upon certain conclusions are discussed in their proper places. So is the effect of »hard seeds» which has introduced disturbances in some cases before the technique to counteract it was fully developed.

In the years 1942—1948 the material was grown at the experimental farm Ugerup, belonging to the Swedish Seed Association, on light sandy soil, more or less rich in lime. It took a few years before we knew the soil of the farm, and in the first years the material was

not very well developed which must to some extent have influenced the detection of mutants. In 1945, 1946 and 1948 the material was very well developed, however, and a number of mutants observed.

For reasons of economy, in 1949 the material was grown on a farm about 5 kilometers south of Svalöf, on sandy soil. In spite of the soil being inoculated with the proper bacteria no bacterial tubers were developed. The development was intermediate, and the observations on mutations fairly reliable. In 1950 the material was sown on another farm in the same vicinity; this year a considerable part of it was destroyed by sand storms, the surviving part was poorly developed, and only few observations could be made this year. In 1951 the material was again sown at Ugerup, the plants grew vigorously and quite reliable observations were made.

Other relevant details of the experimental methods will be discussed when dealing with the results which they may influence.

III. EXPERIMENTAL RESULTS.

1. VIABILITY OF THE X-RAY TREATED SEED.

Tables 1 *a*, 1 *b* and 1 *c* summarize the results concerning the lethal effects of the X-ray treatment. In the first year a number of doses were tested, ranging from 2.500 to 25.000 *r* units, with intervals of 2.500 units. It was concluded that doses around 20.000 *r* would be suitable, but in the next year such doses gave a very low percentage of survival, and from 1944 on doses around 15.000 *r* have been used.

The increase in lethality with increased dose is quite evident, as is also the difference between years in regard to the lethal effects of the X-rays. The differences are so obvious that a statistical analysis concerning significance is considered to be superfluous. The data from 1946, 1947 and 1948 also indicate that the lethal effect of the X-rays may be rather delayed in this material. In 1947 about $^1/_5$ to $^1/_3$ of the seeds gave plants which had emerged on May 30th, but about 5 weeks later most of these plants were dead, while none of the plants from untreated seed had perished. The data from 1949 show a similar picture.

Since no analysis of different properties of the seeds was performed no attempt is made to explain the differences between the years. The material is presented as it stands, and only two features will be discussed in some detail.

Already the results from 1943, when seed not only of strain 8 but also of wild Portuguese bitter yellow lupine was treated, indicated that

the bitter material was far less sensitive to X-rays than the sweet. The indication was fully confirmed in 1944 and 1945, when the viability of the treated seed was much higher in the bitter than in the sweet material in spite of the fact that the percentage of plants from untreated seeds was somewhat higher in the latter material.

A *chi*^{2}-analysis of the absolute data gives values of 34,8 and 196 for 1944 and 1945, respectively. On the other hand, an analysis comparing the difference sweet: bitter at different doses shows the relation between the two types to be essentially the same at all doses in both years.

The difference in sensitivity towards X-rays may be due to differences in the seed-coat — the bitter material has a somewhat stronger tendency towards »hardness» than the sweet strain 8 — or to the existence of alkaloids in the bitter seeds, or, finally, to any other unknown difference between the two types. Since the question of substances »protective» against the X-rays is of considerable interest the eventual effect of the alkaloids in bitter lupine is being followed up. Preliminary laboratory experiments in March—April, 1952, have indicated that bitter blue lupine (*L. angustifolius*) is less susceptible to X-rays than the sweet type of the same species.

TABLE 1 a. *Number of seeds sown and percentage of plants obtained with different doses of X-rays in 1942.*

Dose, *r* units	untreated	2.500	5.000	7.500	10.000	12 500	15.000	17.500	20.000	22.500	25.000
Number of seeds sown	180	194	208	192	190	194	196	206	194	192	192
Percentage of plants	54	15	17	17	18	11	7	3	0,5	2	0,5

TABLE 1 b. *Number of seeds sown and percentage of plants obtained with different doses of X-rays in 1943.*

Dose, *r* units	Sweet lupine				Bitter lupine			
	untreated	17.500	20.000	22.500	untreated	17.500	20.000	22.500
Number of seeds sown	140	196	196	196	140	196	196	196
Percentage of plants	49	0,5	2,0	1,5	86	6,6	1,5	4,6

TABLE 1 c. *Number of seeds sown and number of plants obtained, respectively surviving, with different materials and different doses of X-rays. 1944—48.*

Dose, *r* units	untreated	10.000	12.500	15.000	17.500
Year and material					
1944:					
Seeds sown	196	196	196	196	196
Percentage plants:					
Sweet lupine	83	53	40	14	8
Bitter lupine	78	67	62	23	20
1945:					
Seeds sown	196	196	196	196	196
Percentage plants emerged:					
Sweet lupine	82	—	27	12	11
Bitter lupine	80	—	71	49	37
Percentage plants surviving $^{26}/_{7}$:					
Sweet lupine	82	—	16	6	7
Bitter lupine	80	—	71	45	27
1946: (sweet lupine)					
Seeds sown	56	—	196	392	196
Percentage plants $^{29}/_{5}$	88	—	64	53	39
» » $^{13}/_{6}$	80	—	56	35	24
» » $^{10}/_{9}$	80	—	49	27	18
1947: (sweet lupine)					
Seeds sown	56	—	196	392	196
Percentage plants $^{30}/_{5}$	68	—	36	23	22
» » $^{5}/_{6}$	68	—	34	19	18
» » $^{4}/_{7}$	68	—	14	2	0,5
» at harvest	64	—	13	2	0,5
1948: (sweet lupine)					
Seeds sown	56	—	196	392	196
Percentage plants $^{20}/_{5}$	77	—	84	79	81
» » $^{9}/_{6}$	77	—	81	72	69
» » $^{20}/_{7}$	77	—	78	70	60
» at harvest	77	—	78	68	60

2. FERTILITY IN THE X-RAY TREATED MATERIAL.

A. FERTILITY IN X-1.

As is to be expected, the fertility of the X-1 plants surviving up to the time of harvest is more or less markedly reduced. Exact measurements were made in 1948, including: (1) the number of pods on the main floral axis; (2) the number of ovules in these pods; (3) the percentage of ovules giving (a) well ripened seed, (b) poorly developed seed; (4) the percentage of completely abortive ovules.

The number of ovules per pod is about 5, and this number does not vary significantly from one group of material to another. Comparisons have been made between the standard stock of strain 8, X-1 in 1948 and X-5 and X-6 of two mutants to be discussed later. The total number of ovules in the pods on the main axis is therefore so closely correlated with the number of the pods that the values under (2) above do not offer any additional information of importance. Likewise, the percentage of completely abortive ovules is closely correlated — negatively — with the percentage of well ripened seed, so that the latter gives sufficient information about the fertility. Of the data available, only the number of pods on the main axis and the percentage of

TABLE 2. *The number of pods on the main axis on untreated control plants and on X-1 plants after different doses of X-rays; 1948.*

Material	Number of plants with the number of pods																n	Mean
	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	
Control	—	—	—	—	1	3	2	5	6	9	4	6	2	—	—	—	—	38	18,6
12.500 *r*	—	1	4	4	2	6	9	22	22	26	22	20	12	1	2	—	—	153	18,5
15.000 *r*	3	7	12	8	4	20	27	31	29	29	33	27	18	9	7	1	1	266	17,6
17.500 *r*	2	3	5	2	5	9	9	9	15	12	21	11	10	3	1	—	—	117	18,0

TABLE 3. *The percentage of ovules giving well ripened seed on untreated plants and on X-1 plants after different X-ray doses; 1948.*

Material	Number of plants with fertility percentage between										n	Mean
	10	20	30	40	50	60	70	80	90	100		
Control	—	—	—	—	1	3	10	13	11		38	83,4
12.500 *r*	4	12	16	12	18	13	20	26	31		152	65,4
15.000 *r*	3	31	18	28	32	40	41	52	21		266	62,0
17.500 *r*	2	8	10	15	14	16	25	20	7		117	62,4

ovules giving well ripened seeds are used in the studies of relative fertility.

The data for the standard stock and X-1 in 1948 are given in Tables 2 — number of pods — and 3 — percentage ripe seed.

As is seen from Table 2, there is a very slight indication of a lower number of pods on the main axis in the X-ray treated seed than in the control, but the difference is quite insignificant, as are also the differences between different doses. The analysis of variance gives the following results:

	Sd^2	DF	Var.	Quotient
Total	20.550	573	—	—
Between control and treated	22	1	22,0	<1
Within d:o	20.528	572	35,9	
» treated only	19.965	535	—	—
Between doses	117	2	58,5	1,57
Within d:o	19.848	533	37,2	—

The lethal effect of the X-rays was rather weak in 1948, and the percentage of plants emerged was the same after treated seed as in the controls (Table 1 *c*). Between May 20th and July 20th, however, about $^1/_4$ of the plants after seeds treated with 17.500 *r* died off, half as large a fraction after 15.000 *r* but practically none after 12.500 *r*. There is a significant difference in viability between the three dosages ($chi^2=15{,}4$, $n=2$).

It is interesting to note that in this year the lethal effect of the X-rays has resulted in death in the juvenile stages without any sign of a decrease in vigour in the surviving plants, if this vigour is measured by the mean number of pods on the main axis. Unfortunately the number of control plants studied is rather low, but there is a strong indication of a greater variability among the plants after treated seeds, the quotient between the variances being $37{,}3/15{,}2=2{,}45$, with a P-value not much above 0,001. This would mean that the number of pods was reduced in some plants as an after-effect of the X-raying of the seed, a phenomenon which seems rather likely. The average should then be reached by some of the plants after treated seed having a higher number of pods than the controls. Since there are always a good many more flowers than developed pods such a high number of pods in treated plants may easily be obtained by a better nourishment, caused either

by better spacing for some plants (due to the thinning of the stand by the lethal effect of the X-rays) or, possibly, by the X-ray inhibiting the branching, thus increasing the amount of assimilates available to the main axis. The effects of X-raying the seed upon the development at different stages has to be further studied before it can be considered to be fully elucidated.

If the effect of the X-rays upon the number of pods is questionable and rather small in this material, their effect upon fertility is much more marked, as is seen from Table 3. The decrease in the percentage of ovules giving fully ripened seed is obvious, being about 20 per cent units and about 25 % of the total »fertility-percentage» of the controls.

On the other hand, there is no indication of an increase in the reduction of fertility with increased dose, the analysis of variance giving the following result:

	Sd^2	DF	Var.	Quotient
Total, treated	266.325	534	—	—
Between doses	1.125	2	562,5	1,13
Within »	265.200	532	498,5	—

It is of interest to note that the increase in effect with increased dose, observed on the lethality among the seedlings, has in this case no parallel in an increased effect upon fertility.

B. FERTILITY IN X-2 AND X-3.

In X-2 and X-3 there is a fairly high frequency of partially sterile plants. These are easily observed even by a more superficial inspection of the material. When only one or two ovules per pod are developed into seeds only the corresponding parts of the pod grow to full size, and the pods present a more or less abnormal and distorted shape.

No exact count has been made of the degree or frequency of partial sterility in X-2 and X-3. The frequency varies between different families in these generations, however. In generations later than X-3 only a limited material has been retained, *viz.* the descendencies including mutants which are made the subject of further studies. In nearly all of these cases the plants are normally fertile, as far as can be judged by inspection during growth. That the fertility may be somewhat reduced is shown by the studies reported in the following section.

C. FERTILITY IN HIGHER GENERATIONS OF MUTANT STRAINS.

The number of pods on the main axis and the percentage of ovules giving well ripened seed has been studied in X-5 and X-6 of two different mutations. These, *anthocyanin*$_1$ and *reduced petals*, will be described more in detail and discussed from different points of view in some of the following sections; it will be sufficient to state here that both behave as monofactorial recessives with a small deficit of the recessive in *anthocyanin*$_1$, a considerable one in *reduced petals*. Both recessives are vegetatively quite vigorous in habit, but the recessive of *red. pet.* is completely sterile.

In 1948 the average number of pods on the main axis was $18{,}39 \pm 0{,}73$ for 28 control plants, for 498 plants in X-5 of the mutant *anth.*$_1$ it was $17{,}37 \pm 0{,}23$. In 1949 the values were, respectively, $12{,}22 \pm 0{,}29$ for 139 control plants and $13{,}81 \pm 0{,}15$ for 646 plants from the mutant strain. The plants from the latter include homozygous recessives and homozygous as well as heterozygous normals. There is no difference between these three categories. The differences mutant — control are: $1{,}02 \pm 0{,}77$ and $+1{,}59 \pm 0{,}33$ in the two years. The latter difference is significant, but it may be safely stated that there is no considerable and consistent difference between the mutant strain and the standard in this respect.

In 1948 the 28 control plants had an average percentage of well ripened seed of $82{,}90 \pm 2{,}25$, the 498 plants of the mutant strain had the average $71{,}65 \pm 0{,}27$; the difference is $11{,}25 \pm 2{,}25$. In 1949 the values were $74{,}30 \pm 1{,}10$ for 139 control plants and $67{,}45 \pm 0{,}75$ for 649 plants from the mutant strain; the difference is $6{,}85 \pm 1{,}35$. The difference is highly significant in both years, and there can hardly be any doubt that the mutation *anthocyanin*$_1$ is connected with some phenomenon causing a certain degree of zygotic sterility.

It remains to be found whether and in what way the reduction in fertility is directly connected with the mutation. This problem may be studied in the material of 1949, but the comparisons between the three biotypes must be made indirectly. In some of the X-6 families (progenies from single X-5 plants) which segregated in *anthocyanin*$_1$ all normal green plants were harvested and their progeny studied in 1950 at Ugerup. 30 seeds were sown of each progeny, but since the germination was fairly poor sometimes only a few plants were obtained. It is, therefore, not possible to classify the plants of 1949 quite correctly in homo- and heterozygotes, some of the latter being classified as homozygotes

because of the non-appearance of recessives. Of 128 progenies of normally green plants, grown in 1950, 73 showed segregation, while 55 contained only normal plants. The average percentages of ovules giving well ripened seed in the two groups of mother plants were 70,15 and 69,80, respectively, and there is no indication whatever of a difference between heterozygotes and homozygotes.

From another section of X-6 all recessive plants were selected and their genotype controlled in 1950, showing that they had been correctly classified with few exceptions. The average for ripe seed in per cent of ovules for the 309 recessives was 68,45, 212 normal plants (homo- and heterozygous) from the same part of the field had the average value 64,50. There is a difference, but the analysis of variance shows it to have a very low degree of significance.

It may, therefore, be stated that all three biotypes in regard to the mutation *anthocyanin*$_1$ have the same reduction of fertility. In 1948 it was thought that different X-5 families showed different degrees of fertility. Some of the most fertile and some of the least fertile ones were selected for the cultures in 1949. There was no correlation whatever between 1948 and 1949 as regards fertility, neither if the family means of 1948 were correlated with the means of the total progenies of the same families in 1949, nor if the individual plant values in 1948 were correlated with the means of their progenies in 1949. It seems quite evident, therefore, that the sterility observed in this case has no direct connection with the mutation.

The mutant *reduced petals* was extensively studied in the same years and generations as *anthocyanin*$_1$. Some of the data will be presented in section 7; here it may be sufficient to state that no difference at all was found between the normal plants in the mutant strain and the control, neither as regards the number of pods on the main axis nor in fertility percentage, and this holds true of an extensive material from both years.

3. TYPES OF MUTATIONS AND SOME OUTSTANDING PROPERTIES OF SOME OF THE MUTANTS OBSERVED.

With the poor development in some years it is quite obvious that by far not all mutations have been observed or registered. Besides the common occurrence of partial sterility in X-2 and X-3 there is another common phenomenon which has been studied only more superficially, *viz.* the occurrence of more or less dwarfed plants, usually with the

dwarfing accompanied by more or less distinct characteristics, indicative of a general disturbance of the balance. These dwarfs appear rather irregularly; in some cases normal sibplants have been tested by growing their progeny, but in no case studied there has been even a semblance of normal Mendelian segregation. It is assumed (although the proof, unfortunately, cannot be brought) that the dwarfing is a result of meiotic disturbances which result in a certain number of zygotes with serious defects; in other words, the dwarfs are supposed to be only »milder» instances of the zygotic lethality discussed in section 2. In some of the mutant strains which have been kept for several generations with or without a more detailed study, dwarfs appear once in a while even in late generations. One such strain is *anthocyanin*$_1$ which also showed a general reduction in fertility.

No minor mutations, nor any which influence only physiological characters without having correlated morphological effects, have been recorded in the material. In the following paragraphs a brief enumeration and description is given of the mutants so far recorded.

The main purpose of the work was to find mutations which ripened earlier than the standard stock variety. Earliness may possibly be said to be a strictly physiological mutation, but it is most certainly morphologically easily observed — especially earliness in flowering. In yellow sweet lupine 10 different mutations towards early flowering have been observed, in the bitter material two more. In some cases the earliness at flowering time is correlated with early ripening, in other cases there hardly seems to be any such correlation at all. The work with the early mutants is continued on a comparatively extensive scale, mainly for practical purposes, but also some studies of a more theoretical interest are made. The results will be separately published when they are more definite, and here only the further fact may be stated that some of the early mutants have already been tested in yield trials with very promising results, there being no indication that the mutants are less productive, in green bulk or in seed, than the standard.

The most common type of mutants is the chlorophyll-defective. 21 of these have been denoted as *chlorina*, *i. e.* there is some amount of chlorophyll and the homozygotes are viable, although more or less reduced in vigour. The degree of chlorosis and of reduction in vigour varies, in some cases it is apparent only during more or less brief periods during development. One of the *chlorina* mutants, giving approximately a 3 : 1 segregation, is at the same time a semi-dwarf (*chl 6* in Fig. 1 *a*); another type, used by HAGBERG (1952) in his studies on

Fig. 1 *a*. Normal (left) and *chlorina 6* (right).
Fig. 1 *b*. *Chlorina 1* between normal (left) and *chlorina 6* (right).

vicinism, is intermediate between *chl 6* and the normal (Fig. 1 *b*). Finally, the *chlorina* occurring together with the »single stem» mutant (see below section 5) hardly reduces the plant size at all. — Two *chlorinas* observed for the first time in X-4 in 1951 were normal green in the rosette leaves, but the apical leaves of the stem were slightly chlorotic. In one of these types the discoloration was evenly distributed, in the other it imitated a mosaic disease. In both cases the discoloration was fairly slight but nevertheless the plants were very weak and produced only a few seeds. — Still another *chlorina*, discovered in X-4 in 1951, showed a peculiar growth in the rosette stage, the leaf-stalks not being practically horizontal as in the normal, but elevated at an angle

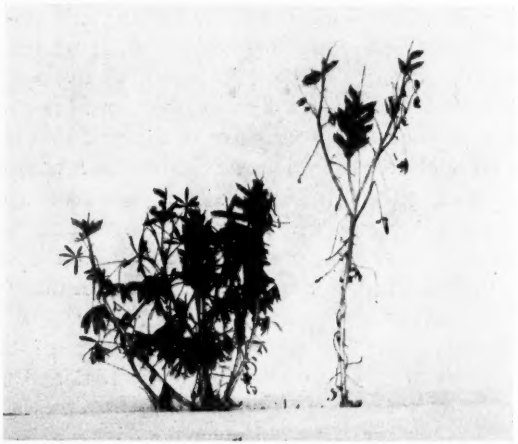

Fig. 2. Normal (left) and a mutant with changed mode of growth and branching (right).

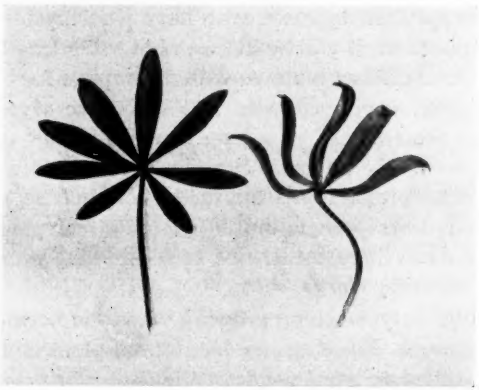

Fig. 3. Normal (left) and a mutant with abnormal leaflets (right).

of about 45°. — Two of the chlorophyll deficient mutants have been designated *xantha*, the loss of chlorophyll being seemingly total and the homozygotes not viable under normal field conditions.

Another type of mutation which has appeared with a somewhat astounding frequency involves an increase in the anthocyanin colouring of the plant, partial or total. The first of these mutants observed has been mentioned already in other connections and will be further dis-

cussed in a couple of sections. Ten other anthocyanin mutations have been recorded, bringing the total of this type to about half that of the total number of chlorophyll deficient mutants. The anthocyanin mutants have not so far been crossed with one another, and some of them may represent the same locus. If so, they are at any rate »alleles», since not two of them are identical morphologically. All the mutations influence the anthocyanin in the leaflets in the rosette stage, some also the colour of the leaf-stalk but others not; some have a marked effect on the apical part of the main axis, others not; in *anth.*$_1$ the colour of the leaves is of a nuance towards the blue end of red, in other mutants the colour is more bright or even orange red.

Six mutations influence the mode of growth of the plant by reducing the duration of the rosette stage. The first one was found in bitter lupine, it was rather dwarfed with miniature leaflets and a slender stem; a very similar type has later on been found in sweet lupine. A third one is the type shown in Fig. 2, which has occurred in a triple mutation; it will be dealt with more in detail in section 5.

One mutant shows the deformation of the leaflets shown in Fig. 3. Another, rather peculiar, type seems to have partially lost the negative geotropism of the stem; it will be discussed at some length in section 6.

There are four distinct mutants with a complete functional sterility in the homozygote, combined with full vegetative vigour. In one of these the petals are reduced, of a pale greenish yellow colour and the flowers never open. The pollen is classified as good to 95 % if studied in Belling-glycerine preparations, but the pods fall off soon after flowering and no seeds have been found on thousands of plants. The other three sterile mutants here mentioned have seemingly normal flowers but are also completely sterile.

All these sterile types go on growing when the normal plants have finished their growth. Fig. 4 shows one normal plant and one recessive of the mutant called *reduced petals* at the time of ripening of the former. The shape of the petals is not visible, but the sterility and the luxuriant vegetative growth are evident. One of the steriles of this type appeared in the same X-2 family as the one with *reduced petals*, just mentioned, and it has quite normal pollen. These two mutants, nevertheless, seem to be quite independent, since they segregated in X-3 progenies from different X-2 plants and have not later occurred in one and the same family. Of the two other sterile types one has pollen that can not function, and the anthers do not dehisce; the fourth sterile mutant was not studied in this respect.

Fig. 4. Normal (left) and the completely sterile mutant *reduced petals* (right).

In this connection it should be mentioned that before the X-ray treatments were applied, a spontaneous mutant with lemon yellow flower colour was observed (*cf.* HACKBARTH, 1938).

4. FREQUENCY OF MUTATIONS AFTER DIFFERENT DOSES OF X-RAYS.

During the years 1944—1948 a total of some 3.500 seeds of sweet lupine, strain 8, have been treated with X-rays, in doses of 12.500, 15.000 or 17.500 *r*. The number of seeds treated with each dose, the number of X-1 plants giving viable seed and the number of X-1 plants

TABLE 4. *Number of seeds treated with different doses, number of X-1 plants obtained and number of X-1 plants giving recorded mutants, absolute and relative; 1944—1948.*

Dose	Seeds treated	X-1 plants		Mutants in ‰ of	
		harvested	mutants	seeds treated	X-1 plants harvested
12.500	980	327	15	15,3	45,9
15.000	1568	404	35	22,3	86,6
17.500	980	168	6	6,1	35,7
Total	3528	899	56	15,9	62,3

giving recorded mutants are given in Table 4, together with the percentage of mutated X-1 plants, counted on the basis of seeds treated and plants obtained.

During most of the years in which X-rayed material has been studied, controls of the standard stock of strain 8 have been interspersed in the material, usually at the rate of one control row to every ten rows of X-rayed material in X-1 to X-3 or X-4. These controls have been studied as carefully as the different generations in the X material, any deviating plants have been harvested, and we may safely maintain that mutants of the types recorded in the treated material would have been recorded to the same extent in the controls. In the years 1945 to 1951 control rows with in all 15.308 seeds have been sown. The plants have not been counted, but the average stand is a little more than $^2/_3$ as many plants as seeds sown, which would mean that about 10.000 to 12.000 control plants have been studied. Among these one *chlorina* and one *anthocyanin* plant have been recorded. With 25 % vicinism it is not at all sure that these two plants are spontaneous mutants, but their number, two in 10—12.000, represents a maximum value of such mutants, and there can be no doubt whatever that practically all of the 56 mutants recorded in the X-ray material are caused by the X-ray treatment.

The percentage of mutating X-1 plants is higher after 15.000 *r* than after 12.500, 17.500 *r* again giving a lower rate. A chi^2-analysis gives P-values only slightly lower than 0,05, whether all three doses are compared at once or the medium dose separately with the highest and the lowest ones. The relation between dose and rate agrees with what is found in other species such as barley (GUSTAFSSON, 1947); the significance being rather slight there is no reason for any detailed discussion.

Some of the X-1 plants have given rise to more than one mutant. These »double» or »triple» mutant cases are (a) *reduced petals*+sterile with normal flowers; (b) *chlorina*+*anthocyanin*; (c) *anthocyanin*+complete sterility; (d) deformed leaflets (Fig. 3) and *xantha*; (e) mode of growth+*chlorina*+complete sterility. Thus, the total number of mutants recorded from 56 plants is 62, giving a frequency of 0,069 mutations per harvested X-1 plant. With this frequency two mutants after one X-1 plant should be obtained with a frequency of 0,0048 or 4—5 times in 899 plants. Three mutants should occur in the frequency 0,0003, or less than once in 899 plants. The cases of double mutations are just as many as expected, the single triple mutant cannot as such

indicate any tendency for mutants to group themselves in certain X-1 plants.

The mutants in pairs listed above under (a) to (d) have also behaved as quite independent. The triple mutant mentioned under (e) behaves in a quite different way and it will be treated more in detail in the next section.

5. A CASE OF THREE DIFFERENT BUT LINKED MUTATIONS IN ONE X-1 PLANT.

In one X-2 family in 1947 three plants were observed which were slightly *chlorina* in colour and had a mode of growth different from that of normal yellow lupine. The rosette stage when only basal leaves are developed, which is rather extended in the normal type, was considerably abbreviated, and the main stem began to grow upwards about as rapidly in this mutant type as in the blue lupine. Later on, it was also evident that the mode of branching differed: the normal yellow lupine has a number of basal branches and some which are more or less apical, the mutant (Fig. 2) has none or only a couple of basal branches but several more or less apical; even the mode of branching imitates, when most typical, the blue lupine. At the time of harvest, all three mutant plants in X-2 were completely sterile, and it was thought that we had encountered a mutant with a pleiotropic effect on mode of growth, chlorophyll and fertility. Seven plants, which were annotated as »normal», were harvested. In X-3 three of these gave only normal offspring, and they may have been normal homozygotes. One plant gave only 5 daughter plants, 4 of which were mutants. It seems probable that the X-2 plant in this case had been a mutant in *chlorina* and mode of growth, the one normal plant in its progeny resulting from vicinism. The remaining three X-3 families segregated in normal and mutants. All mutants in regard to mode of growth were still *chlorina* and *vice versa*, but not all of these were sterile, while in one family one plant with normal growth and colour was sterile.

In later generations it has been possible to separate also mode of growth and *chlorina*, and it is evident that the three mutations are to be found in definitely separate loci. In segregating families so far studied in detail, the segregation in regard to each of the mutants has been as seen in Table 5. As symbols are used *chl* for *chlorina*, *bbr* for the mode of growth (no basal branches) and *st* for sterility.

TABLE 5. *Segregation in regard to the mutant types »chl», »bbr» and »st».*

Mutant type	Normal plants	Mutants	Total
chl	478	118	596
bbr	266	59	325
st	353	55	408

These figures do not give the exact segregations, since there are several sources of error. The vicinism will tend to decrease the number of recessives (*cf.* HACKBARTH, 1951). On the other hand, there are some families which may be either progenies of heterozygotes with an unusually high number of homozygous recessives or progenies of mutants with an unusually high number of vicinists. Finally, since in most cases only about 10 plants are obtained in each progeny from 15 seeds sown, a number of heterozygotes will not show any mutants in their progeny, which tends to decrease the relative frequencies of normals in the segregations. On the whole it seems safe, however, to state that the three mutants behave as essentially monofactorial with a more less marked deficiency of recessives — a rather common type of occurrence in material of this type.

That the linking together of the three characters in the X-2 family, where they were first observed, was not a coincidence is evident from the hybrid segregations shown in Table 6.

TABLE 6. *Dihybrid segregations in the mutant types »chl», »bbr» and »st».*

	Bbr Chl	*Bbr chl*	*bbr Chl*	*bbr chl*	Total
Found	167	9	5	40	221
Expected if free combination	137	39	35	10	221
	Bbr St	*Bbr st*	*bbr St*	*bbr st*	Total
Found	136	19	28	15	198
Free combination	128,4	26,6	35,6	7,4	198
	Chl St	*Chl st*	*chl St*	*chl st*	Total
Found	177	21	44	15	257
Free combination	170,3	27,7	50,7	8,3	257

The linkage between *bbr* and *chl* is beyond doubt, that between *bbr* and *st* gives a chi^2 of 12, with $P < 0{,}001$, the linkage between *chl* and *st* is the weakest, with chi^2 only 8,18, $P < 0{,}05$.

There can be no doubt that we have to do with three different mutations in three distinct and separable loci, but that these loci are either in one and the same chromosome or that, if they are in separate chromosomes, the mutations involve such chromosomal changes that the chromosomes in question are brought together in the meiosis

The chances to find 3 mutations in one X-1 plant are only about 3 in ten thousands or less than $^1/_3$ % in the present material; the chance that

TABLE 7. *Percentage of ovules giving ripe seed in different biotypes in X-3.*

Biotype	% fertile ovules					Mean
BbrBbr ChlChl StSt	59,3	64,8	64,0	86,5		68,7
Bbrbbr ChlChl StSt	69,9	81,3				75,6
BbrBbr Chlchl StSt	86,0	85,2	61,1	88,8	63,9	
	76,3	85,3	70,9	50,8	67,5	73,6
BbrBbr chlchl StSt	50,0	80,3	79,9	82,6		73,2
BbrBbr ChlChl Stst	75,8	68,8	44,0			62,9
Bbrbbr Chlchl StSt	86,0	75,2				80,6
BbrBbr Chlchl Stst	54,4					54,4
Bbrbbr Chlchl Stst	78,7	83,1	88,4	83,2		83,4

they should occur in one out of 52 chromosomes is infinitesimal. The linkage must, therefore, involve either some kind of a chain reaction in one chromosome or some other complicated phenomenon. Unfortunately, the small size of the lupine chromosomes again prevents a further study of a case which seems to have some promising features.

The fertility in different homozygous and heterozygous biotypes should be of interest in this connection. The percentage of ovules giving well ripened seeds was determined on 30 plants in segregating families in 1948. Only a few plants of each biotype were involved, the data are published here to complete the record as far as possible (Table 7).

6. MODIFICABILITY OF THE MUTATIONS.

It has been known for a long time that even some rather drastic mutations are susceptible of considerable modification by environmental factors, or by modifying genes. The whole question of the

plasticity of the biotype seems to be of such a great interest, however, that we devote a special section to a report on our observations in this respect.

No systematic experiments have been made with the *chlorina*, *xantha* and *anthocyanin* mutations in regard to their behaviour in different light intensities. Some *chlorina* and *anthocyanin* mutants have been grown in greenhouses in winter, however, and it was found to be impossible to classify the seedlings. One of the *xantha* mutants, which had proved completely lethal in the years 1949 and 1950, survived partly, without setting any seed, in 1951, supposedly due to the shading offered by the neighbouring plants, which grew vigorously in that year, perhaps also because of the unusually favourable growth conditions of that year.

The mutant described as having lost the negative geotropism offers a striking instance. It was observed towards ripening in X-3 in 1948. The segregation was of the usual type, monohybrid but with a deficit of recessives. The recessives were very typical — unfortunately, no photograph was taken in 1948. The stems were lying down on the soil. It was obviously not a question of a weak stalk, since the stems were not bent over just above the soil surface and for the rest straight; instead, they were lying down »in a tired manner», the stems curving around on the soil. The fertility was considerably impaired, but viable seed was obtained from a number of recessives. The plants grown from these seeds in 1949 could in no way be distinguished from normals, nor was there anything of the characteristics of the mutation to be seen in 1950; indeed we believed that some mistake had been made and that the mutant had been lost. In 1951, however, the mutation became again visible. Towards ripening the stem began gradually to become curved and did not grow straight upwards. The leaves also showed some abnormality. The characteristics of the mutant were, however, by far not so striking as in 1948. We have here a case of a mutation that within the comparatively narrow variation in environment offered by growth conditions in different years and places within a limited region may vary from a completely normal exterior, over a slightly aberrant mode of growth to a strikingly abnormal development, leading to semi-inability for propagation and even to a marked decrease in vegetative competitive ability.

Finally the first of the anthocyanin mutations found has been the subject of some special studies of the modificability in the development of the anthocyanin colour.

The mutant in question segregates fairly regularly; one count in a number of segregating families gave a total of 587 normal to 168 anthocyanin, as against 566 : 189 by quite regular 3 : 1 segregation. The deficiency in recessives is not more than may be accounted for by the vicinism.

In the late summer of 1948 it was observed that the anthocyanin colour, which had up to then been noted only in the early rosette stage, was very evident in the upper part of the main axis. At the same time there was a marked relationship between this colouring and late development, and a considerable material was harvested in order to study the problems involved.

The relationship between anthocyanin and lateness was found to be caused by an evident tendency for the plants with anthocyanin to have a more »hard» seed-coat — no further time or space is spent on this relationship.

When the plants were classified according to the »top»-anthocyanin occurring especially in the upper part of the main axis, it was found that this classification did not coincide with the classification made in early summer for »rosette»-anthocyanin. The character of the individual plant had not been noted, but the segregation normal : anthocyanin was different in the two classifications. The study therefore was so planned, as to answer whether the two appearances of the red colour were caused by one or by two mutations. A considerable amount of material has been studied, the conclusions are here summarized.

The anthocyanin is most clearly evident in spring and early summer and in late summer. Between these two times the difference between normal and mutant may under certain conditions be quite indiscernible; in some instances it may be observed in a majority of the plants. It seems fairly safe to assume that the differences in the strength of the colouring have something to do with the well known fact that the appearance of anthocyanin in plants is often connected with low temperatures.

One and the same mutated factor causes the appearance of anthocyanin in both the rosette stage and the top stage; there cannot be any doubt about this main fact. But, on the other hand, the two manifestations are differently modified, both by environmental factors and by genetical modifiers.

The former statement is proved by the fact that the classification, as controlled by the growing of progenies, is sometimes more reliable in the rosette stage, sometimes in the top stage. As the same set of

conditions do not prevail in spring and in autumn, it is perhaps not correct to say that the same environment may influence the two manifestations differently, only that these are differently developed, relatively, in different years.

That the relation between the two manifestations may be influenced by selection is shown by the following facts. In 1949 most of the plants were noted as anthocyanin coloured both in the spring and the autumn or at none of these times. Some exceptions occurred, however, and in 1950 the progenies were sown after 24 plants noted as anthocyanin in the rosette stage but as green in the top stage, and after 36 plants with the opposite irregularity: green in spring and red in autumn. Five, respectively three of these plants gave only green progeny and were most probably misclassifications in 1949; even the normal type is not free from anthocyanin.

In 1950 only one single plant in the progenies here discussed was classified as green in the spring but as anthocyanin coloured in the autumn. Disregarding this plant and the ones classified as normal green (probably vicinists), we may divide the rest of the plants into *aa*, annotated as anthocyanin coloured at both times, and *ag*, anthocyanin in spring, green in autumn. The relations between these two groups in the progenies of plants belonging in 1949 to the groups *ag* and *ga*, respectively, were:

Type of plant in 1949	Number of plants in 1950	
	aa	*ag*
ag	32	55
ga	128	47

Chi^2 for homogeneity is 32,23, with P far less than 0,001, and it is evident that the tendency not to show the anthocyanin colour in the autumn of 1950 was relatively more common in the progenies of plants which did not show it in the autumn of 1949.

The proof of the hereditary nature of the difference may be carried one step further. The mother plants of the plants chosen in 1949 as *ag* or *ga* were classified, in the autumn of 1948, in regard to their »top-anthocyanin».

Of the 19 true anthocyanin recessives in 1949, classified as *ag* this year, 10 had been derived from mother plants which were classified as green in the autumn of 1948; of 33 true recessives in 1949, classified as *ga*, also 10 had mothers, classified as green in 1948. The difference

points in the same direction as before, but is not significant. If the families of 1950 are grouped according to the classification of the grandparental plants in the autumn of 1948, and the classification in *aa* and *ag* determined for each group separately, the following data are obtained.

	Number of plants in 1950	
	aa	*ag*
Parent plant 1949 *ag*		
Grandparent »top-anth.»	16	18
» »top-green»	16	37
Parent plant 1949 *ga*		
Grandparent »top-anth.»	101	22
» »top-green»	27	25

In both groups the tendency for the grandparental character to prevail in 1950 is evident; the data for the smaller group give no significance, in the greater group chi^2 for homogeneity is 16,83, with $P<0,001$.

There can be no doubt that the strength of the effect of the mutant factor upon the colouring of the apical part of the main axis in the autumn may be influenced by selection and therefore must be at least to some extent influenced by heritable factors. It is impossible to decide, however, whether or not this is inherent in the mutation as such. With the high degree of vicinism the original strain 8 probably was not a pure line, and the effect of the selection may perhaps be most easily explained as a selection of genes, heterozygous in the X-1 plant in question even previous to the X-ray treatment.

7. THE RELATION BETWEEN NORMAL HOMOZYGOTE AND THE HETEROZYGOTES OF SOME MUTATIONS.

When the work here reported was under way the results of GUSTAFSSON (1946), JONES (1945) and others made the question of so-called monohybrid heterosis a rather actual one, and it was obvious that we should look out for any signs of this phenomenon in our material. In the section discussing the sterility in X-1 and following generations the data obtained on material belonging to the anthocyanin mutation have already been presented; they do not indicate any superiority for the heterozygote.

In 1948 there were 112 X-5 progenies from normal plants in families segregating 1947 for *reduced petals*, 57 of these had only nor-

mal plants, in 55 there was a segregation in 515 normal to 120 recessives. There was an indication of a differentiation between the descendencies of different X-3 plants in regard to the proportion of recessives. This matter was studied again in 1949, but there was no true differentiation; the segregating families this year gave 1218 normal to 285 *red. petals*. The segregation, thus, is the usual one, monofactorial with a deficit of recessives.

In 1948 there was on an average 11,53 plants per row in the segregating families, 11,12 in the non-segregating ones. The difference was not significant but there was an indication of superior vitality in the seed from the heterozygote, and the matter was followed up. The number of pods on the main axis and the percentage of ovules giving ripe seed were determined on a number of plants in 1948 and their progeny grown in 1949. This year again a considerable number of counts were made and the progenies grown in 1950. Especially in 1950 the development was poor and there was a low number of plants in each progeny. A considerable number of heterozygous plants must have been wrongly classified as homozygotes. If there had been any considerable difference between homozygotes and heterozygotes, this would have been still apparent in the comparison between on one hand a group of certain heterozygotes, on the other a mixture of hetero- and homozygotes, with the latter more or less prevailing in number.

As is seen from Tables 8 and 9, there was no indication at all of a monohybrid heterosis in this character. The data are published here, nevertheless, since one of the authors (HAGBERG) is just at present writing a paper on monohybrid heterosis.

IV. DISCUSSION.

The main purpose of this paper is to present the empirical data obtained; some details have been discussed in the several sections. A couple of further points of interest may be brought to attention here.

The chromosome number of the yellow lupine, $2n=52$, would seem to indicate polyploidy. Nevertheless, the frequency of mutants obtained by X-rays is more similar to such frequencies found in diploids, which is true especially of the comparatively high number of chlorophyll deficient mutants found. FRÖIER (1946) found a much higher frequency of such mutants in the diploid species of wheat than in the tetra- or hexaploid species, and MÜNTZING (1942) reports that

TABLE 8. *The number of pods on the main axis on normal homozygotes and heterozygotes in families segregating in the mutation »reduced petals»; 1948 and 1949.*

Material	Number of plants with the number of pods																n	Mean
	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31		
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32		
homozygotes, 1948	1	—	—	3	4	7	10	15	16	20	14	18	10	3	1	—	124	18,8
heterozygotes, 1948	—	—	2	1	6	9	18	18	19	39	36	27	17	11	4	1	206	19,6
homozygotes, 1949	—	5	15	22	90	107	116	72	42	26	12	3	—	—	—	—	510	12,9
heterozygotes, 1949	—	—	10	17	40	38	49	33	14	7	3	1	—	—	—	—	212	12,5

TABLE 9. *The percentage of ovules giving well ripened seed on normal homozygotes and heterozygotes in families segregating for the mutation »reduced petals»; 1948 and 1949.*

Material	Number of plants with fertility percentage between										n	Mean
	0	10	20	30	40	50	60	70	80	90	100		
homozygotes, 1948	—	1	—	2	2	3	5	28	39	45		125	83,1
heterozygotes, 1948	—	1	—	1	—	8	14	40	63	81		207	84,3
homozygotes, 1949	3	2	5	4	7	16	34	82	175	182		510	82,4
heterozygotes, 1949	—	—	1	1	1	11	16	37	88	57		212	82,4

the frequency of visible chlorophyll mutants was much lower in tetraploid than in diploid barley.

Another interesting comparison concerns the type of chlorophyll mutants. None of the mutants observed in the material here presented have been true *albina*, a few have been *xantha* while most have had more or less of green colour, thus belonging to the *chlorina* type. According to VON ROSEN (1942), no *albina* mutants have been found in peas either, although a number of chlorophyll deficient mutations have been found; all of these have been yellow or light green. Of the present authors, TEDIN can confirm this from his own unpublished experience while working with peas; a few cases of spontaneous chlorophyll deficient mutations were noted, but none of these were pure white. In striking contrast to lupine and peas stand wheat and barley, where *albina* mutants are most common among the chlorophyll deficient ones (FRÖIER, 1946; GUSTAFSSON, 1947).

Certain types of mutants, other than chlorophyll deficient ones, seem to be fairly common in certain species. In barley the more or less dense-eared *erectoides* has been found a fairly great number of times (GUSTAFSSON, 1947; HAGBERG and TJIO, 1950), in wheat the *speltoid* and lax-eared mutants are comparatively common (FRÖIER, 1946; GUSTAFSSON and MAC KEY, 1948). Corresponding to this, mutants with an increase in anthocyanin have been found rather frequently in the lupine, also mutants which flower more early than the standard stock and, finally, mutations changing the mode of growth have been repeatedly found.

It would be of interest to compare the frequency and types of mutants found after X-ray treatment with the spontaneous mutations recorded in lupine. The German breeders have reported a number of »mutations» in yellow lupine. Some of these have been found in populations of bitter lupine and are named »mutants» in acceptance of the generally adopted theory that such recessive types have once originated as mutants. Their frequency is the result at the present time of mutation pressure and rate of selection over an unknown but certainly long period of time and is of no direct interest in this connection. Some mutants have been reported to occur for the first time in the original strain 8, and since this strain is descended from a single plant these cases may reasonably be supposed to be new mutations. White seed-coat has been found in two apparently quite independent instances (TROLL und SCHANDER, 1938; v. SENGBUSCH, 1940); this mutant has not been observed in the present material. A type with

reduced duration of the rosette stage has occurred in strain 8 (HACKBARTH, 1951), besides a couple of similar ones found in population material. These types are more or less similar to a number of mutants found in the present material. A mutant with »sulfur yellow» flower colour may conceivably be identical with the one found without X-ray treatment in the present material and here called »lemon yellow». It is reported (HACKBARTH, 1938) as having been found in five places in 1937. It is impossible, however, to judge whether these cases are independent or not. Nor is the possibility excluded that the material originally obtained in Sweden from Germany may have included a few plants recessive for the mutant gene, although the mutant was not observed until after a few years.

The occurrence of anthocyanin mutations has been recorded in blue lupine (TROLL, 1943) but no cases have been found by us, where they have been recorded in yellow lupine, nor have we found any mention of chlorophyll mutants. This may, however, simply be due to the fact that the German lupine breeders have interested themselves mainly in types which may be of practical value, and no inference can be made from the lack of recorded, spontaneous, chlorophyll mutants. On the other hand, mutations in regard to alkaloid content, non-hard seed or not shedding pods have not been looked for in the material here presented.

Attention may here be called to the occurrence of two distinctly different types of sterility in the X-ray treated material. In X-1—X-3 a partial sterility is a regular phenomenon, and in one case a partial sterility has been found as late as in X-5—X-6 in a mutant strain. In striking contrast stands the absolute sterility observed in four cases, at least three of which segregate according to the monohybrid scheme with a deficit in recessives and at least two of which have seemingly good pollen.

The great modificability of some of the mutants studied has been pointed out. This holds true of the mutant with a partial loss of the negative geotropism, of the chlorophyll mutants as well as the anthocyanin ones and also of the mutations concerning earliness. A striking difference between the frequencies of mutations found in different years is obvious if the material is studied in respect to this detail. The great modificability makes it impossible to know, however, whether this annual variation is due to differences in the true frequency of mutations or simply to the fact that most mutations are more easily detected in one year than in another, and we have, therefore, re-

frained from giving and discussing any data pertaining to the annual variation.

It may, finally, be stated that the induction of mutations with X-rays has proved to be a promising method of practical plant breeding in yellow lupine. The number of early mutants is fairly high in comparison with the total number observed, and all of them are fully vital as judged by the eye; a couple of them have also been tested in yield trials and found to be equal to the parent strain in yield of green matter as well as of grain. Even some of the mutations in type of growth may be of practical value, especially when the lupine is to be used as a second crop in late summer and fall.

SUMMARY.

The paper presents the results from 10 years work with X-ray induced mutations in yellow lupine.

The main purpose of the work was to find practically valuable characters; early mutants have been found several times and these are fully vital, so that the usefulness of the method in breeding work seems beyond doubt.

Some facts of a more theoretical interest have been recorded and the present paper deals mainly with these observations.

Doses of 12.500 to 17.500 *r* units seem to be most favourable, 15.000 giving the highest mutation rate among surviving plants; with 20.000 *r* nearly all X-1 plants die before seed setting.

The sweet yellow lupine shows a higher degree of lethality in X-1 than the bitter type with the same dose; a similar difference has been observed between sweet and bitter blue lupine.

The fertility of the surviving X-1 plants is lower than that of the controls; in the only case studied in detail the increase in dose from 12.500 to 17.500 *r* has not decreased the fertility.

Even in X-2 and X-3 there occurs a great number of partially sterile plants, but their number gradually decreases.

In one mutant strain, studied in X-5—X-6, there was a general reduction in fertility, equal in homozygous normals, heterozygotes and homozygous recessives. In another mutant, studied in the same generations, there was no reduction in fertility of homo- or heterozygous normals, although the homozygous recessive was completely sterile.

Such complete sterility has been observed in four cases, three of which at least behave as monohybrid recessives and two of which have apparently good pollen.

The most frequent type of mutation is a reduction in the amount of chlorophyll, but among 23 such mutations recorded not a single one was completely white.

An increase in the content of anthocyanin has been observed in 11 cases; this being the second most common type of mutant recorded.

A reduction in the duration of the early rosette stage of the development has been found in six cases. Several other types of morphological mutations have occurred, each once.

The total frequency of the mutations recorded is 6,9 per 100 harvested X-1 plants, which certainly is not the total mutant frequency, since a number must have passed unrecorded with the methods used. In all about 10.000 control plants have been grown; two cases of apparent mutations have been recorded, which may or may not be due to spontaneous crossing.

Two mutations from one X-1 plant have been obtained in four cases, three mutations in one case, which does not indicate any tendency for the mutations to cluster together in certain X-1 plants.

The case with three mutations in one X-1 plant is peculiar, however, inasmuch as the three mutations appear obviously in three different but linked loci. That this should be due to chance is practically impossible, but no definite explanation can be offered, since the material, unfortunately, is unfit for detailed cytological studies.

The mutants are mostly greatly modifiable by environment. Especially one case is discussed, where in different years the same homozygous recessive varied from apparently completely normal growth to a total loss of the ability to grow upright, with the result that the seed setting was very strongly reduced; in this year the mutant would have to be classified as a semi-lethal.

In one of the anthocyanin mutants selection for the intensity of colouring was effective; it could not be determined whether the heritable variability was inherent in the mutant strain as such or due to »modifiers» existing in a heterozygous condition in the parent strain.

In two mutant strains the homo- and heterozygous normals were compared in regard to the number of pods on the main axis and to fertility. No monohybrid heterosis was observed.

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POTASSIUM CYANIDE IN THE ALLIUM TEST

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INTRODUCTION.

POTASSIUM cyanide, owing to its quality of being a powerful enzyme poison, has often been made use of in cyto-physiological experiments for depressing the respiratory activity of the cell. Thus it has been employed in experiments on chemical mutagenesis, as an addition to other chemicals, for the purpose of keeping down the catalase activity of the cytoplasm during the treatment. In this connection it is undoubtedly of great interest to analyse the cytological action of KCN. It is important to know the threshold concentrations for various cytological reactions and to determine the highest concentration at which the cells do not deviate from normal behaviour.

Already in 1944 the senior author made several experiments on the effect of KCN on root meristems of *Allium Cepa*. Only a few data from these experiments have been published, *viz.* the discovery that KCN is able to induce a complete and typical c-mitosis (LEVAN, 1945), and certain data concerning the despiralisation effect of KCN (LEVAN, 1949). On this latter occasion two photomicrographs of an *Allium* cell were presented, in which the chromosomes exhibited an extreme type of despiralisation, induced by 12 hours' treatment in 0,05 mol/l KCN. These pictures belong to the experimental series 1 below, and a half-schematic drawing of the same cell is given in Fig. 5 of the present paper.

While staying at the Institute of Genetics, Lund, during the spring of 1952, the junior author worked through the existing slides from these experiments, and, in addition, arranged a supplementary series of KCN treatments. The final analysis of the entire material was made by us in co-operation.

The experimental technique employed is the one usual in the *Allium* test method (LEVAN, 1949). Besides the ordinary stainings in crystal violet, some slides were made with haematoxyline and Feulgen stainings, especially for the despiralisation analysis.

GENERAL OUTLINE OF THE EXPERIMENTS.

Among the six experimental series arranged the one called No. 4 will be omitted here. It dealt with the question whether a long treatment in a weak concentration of KCN has any tendency to make the cells more resistant to the drug. Its results, although in a certain degree indicative, were not conclusive, so it has to be repeated. Series No. 6, made during 1952, was only intended as a corroboration to earlier series. Since its main results were in good agreement with earlier results, it will not be treated in details here, although it is taken into account in building up the general conclusions. Concerning the other series, the following data may be given:

Series 1 (started October 8th, 1944). Concentrations: 1, 0,5, 0,1, 0,05, 0,01, 0,005, 0,001, 0,000'5, 0,000'1 mol/l (everywhere in this paper concentrations will be given as mol/l). pH was falling from 10,5 to 9,0 in the region 0,1—0,005; in the lower concentrations it was about 7,7. Root material was fixed after 12 hours and 2 $^1/_2$ days.

Series 2 (started October 20th 1944). 14 concentrations from 0,025 to 0,000'001. Fixations (only down to 0,000'01) were made 18 hours, 2, 3 and 6 days after the start of the treatment.

Series 3 (started October 27th, 1944). This series was intended for analysing the development of the lethal changes. Concentrations: 0,1, 0,075, 0,050 and 0,025. pH $>$ 10 in all of them. Fixations after 7, 15, 30 minutes, 1, 2, 4, 8 and 12 hours.

Series 5 (started November 7th, 1944). Further analysis of the lethal zone. Concentrations: 0,075, 0,066, 0,058, 0,050, 0,041, 0,033 and 0,025. Fixations after 12 and 22 hours.

MACROSCOPIC OBSERVATIONS.

Loss of turgor of the roots is generally taken as indication of lethality. As usual in strong alkaline treatments, the soft roots often take on a peculiar transparent appearance. A certain degree of maceration of the root tissues is brought about by strong KCN solutions. The turgor of the roots was lost under the following conditions:

Concentration	Time till loss of turgor
0,1	15 minutes
0,075	30 »
0,05	2 hours
0,025	12 »
0,01—0,000·5	2 days
0,000·25	5—7 days

From 0,000·1 down the roots kept turgescent all through the experiments.

YAMAHA (1927), in his extensive studies on the effect of various chemicals on *Vicia faba* roots, also included KCN in his experiments. His time of treatment was only 2 hours, followed by 1 hour's rinsing. He reports 0,063 to be the lowest lethal concentration. RYLAND (1948) in *Allium* roots finds that 25 hours in 0,05 bring about extensive necrosis, while the same time in 0,005 only causes death of certain cells in the tissue.

C-tumours were observed in our experiments in 0,000·05 and 0,000·025 after 3 days. Also in 0,000·1 a certain c-tumour growth occurred, although the toxicity of this solution did not allow real tumours to be formed. There appeared, however, irregular turnings and bendings in the elongation region of the roots, a condition which is known to correspond to c-tumours in less toxic treatments. In 0,000·025 the c-tumour reaction is only temporary: the root tip grows on, so after some time the original c-tumour will be found as a small swelling far off from the tip of the root. In 0,000·01, and lower concentrations, no c-tumours are formed: the roots are growing out. From 0,000·005 they grow as rapidly as the controls. No special attention was directed to the question whether any stimulation of the length growth occurs in any concentration zone of KCN.

LETHAL ZONE (FROM 0,025 UPWARDS).

The zone of the strongest concentrations in our experiments is dominated by very characteristic cytological changes. Its general characteristics are due to the strong alkaline effect of KCN, and similar effects may be induced unspecifically by any alkaline solution. The stainable substance is washed out from the nuclei and the chromosomes, with the result that sections of roots treated with 1 or 0,5 KCN are extremely difficult to stain. RYLAND (*l. c.*) reports this difficulty in the strongest concentration used by her. Mordanting before staining is of little use. Even Feulgen often gives negative results, indicating that most of the DNA has disappeared from the chromosomes. It is often noticed that different regions of the same root respond differently in this respect, the meristem usually keeping some degree of stainability longer than older parts of the root. Not seldom, however, arbitrarily situated islands of tissue may show good staining capacity in tissues otherwise unstainable.

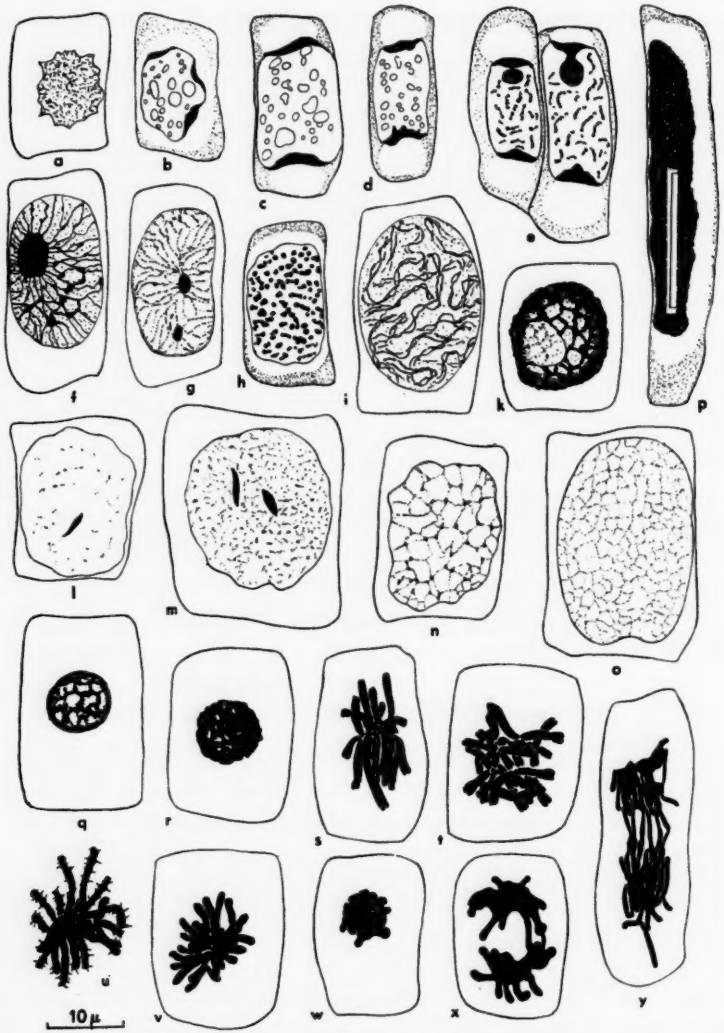

Fig. 1. *a—p*: the influence of KCN solutions of the lethal concentration zone on resting nuclei, *a*, *f—k*: of the meristem, *b*: subepidermal layer above the meristem, *c—e*: of the plerome, *l—o*: old parts of the periblem, *p*: an old vascular cell. *x—y*: pycnotic anaphases from the same type of treatment. *q—w*: the influence of KCN solutions of the upper c-mitotic zone, *q—r*: pycnotic resting nuclei, *s—w*: various responses of metaphase chromosomes. Dosages: 0,1 mol/l, 7 min: *y*; 15 min: *a*, *x*; 30 min: *h*, *m*, 1 h: *c*, *o*; 2 h: *b*, *l*; 0,075 mol/l, 8 h: *e*; 0,05 mol/l, 12 h: *d*, *f*, *g*, *i*, *n*, *p*; 0,025 mol/l, 18 h: *k*; 0,01 mol/l, 12 h: *q—w*. — ×1150, *p*: ×400 (the length of the scale in *p* is 50 μ).

Strong alkaline treatments induce an extremely characteristic picture: the nuclei swell up immensely, and the plasm is pushed outwards towards the cell walls. Between the nuclei and the plasm there is a big vacuole surrounding the nuclei on all sides. This condition is made especially clear in haematoxyline stain or in phase contrast (Fig. 1 *b*, *h*, *p*). We do not know whether this very striking condition is present before the fixation, or if it is effected by a shrinkage of the nucleus brought about by the fixation.

A somewhat different type of vacuoles in the plasm occurring after lethal treatments is represented in Fig. 1 *c*—*e*. A similar cell is photographed by YAMAHA (*l. c.*) in his Fig. 113. Especially in the oblong cells of the plerome it often occurs that the nucleus swells up filling out the central part of the cell. At both ends outside the nucleus there is a big vacuole in close contact with the nucleus. The most striking feature is, however, that most of the chromatic material of the nucleus has been gathered to the nuclear membrane close to the vacuole. If there are nucleoli present, they are usually situated in contact with these chromatic disks. It may give a striking appearance to entire tissues, when all cells show this type, the two chromatic disks at the »poles» of each cell giving an impression of numerous anaphases. We usually refer to them as »pseudo-anaphase» nuclei. Also in this case we are unable to decide whether the vacuoles are due to a fixation effect, originating in the nucleus suddenly pulling away from the ends of the cell at the moment of fixation after having earlier completely filled up the unfixed cell. However, these »pseudo-anaphase» cells constitute a very characteristic feature in strong KCN treatments as well as in other strongly alkaline treatments. They are frequent, for instance, at a dose of $0{,}1$—$0{,}05$ KCN for 1—12 hours.

The structure of the nuclei is strongly affected by the lethal KCN treatments. If it is possible at all to stain the nuclei, it will be seen that the chromatin is dispersed into many fragments scattered all through the nucleus or pressed against the nuclear membrane. There may be formed either many rounded lumps as in Fig. 1 *b*—*d*, numerous irregularly shaped grains (Fig. 1 *h*), loose pieces of fibres (Fig. 1 *e*), or many thin threads, in which case the original nuclear structure may be seemingly better preserved (Fig. 1 *f*, *g*). Especially in older parts of the root at some distance from the tip, the nuclei are very large and their chromatin is very finely dispersed into almost invisible tiny fragments (Fig. 1 *l*) or into a network (Fig. 1 *m*—*o*). In the very large cells of the vascular tissue the big nuclei may contain an amorphous mass

of chromatic material, often quite deeply stained. The nuclei of these cells form long rod-like structures (Fig. 1 *p*), often exceeding 100 μ in length. In these cells where the chromatin is so strongly destroyed the nucleoli are nevertheless often to be seen (Fig. 1 *l*, *m*). They are, however, much smaller than normally is the case.

When the resting nuclei take such types as described, the mitotic chromosomes are extremely interesting. They rapidly lose their chromatic properties, *i. e.* the nucleoprotein envelope, called matrix, disappears. During the process of being deprived of their matrix the chromosomes are elongated, eventually extremely so. We bring about an artificial despiralisation of the chromosome. Fig. 1 *h* shows a prophase in a swollen nucleus the chromosomes of which are despiralised. In the last section of the present paper the despiralisation phenomenon will be analysed in somewhat more detail.

The experimental series 3 gave an opportunity to follow the development of the lethal changes step by step. A few data from this series will be mentioned here. In the highest concentration of this series, 0,1, already a treatment of 7 minutes gives certain changes. Resting nuclei, still of normal size, take on a coarser structure than normally. They are deeply stained. Division stages are more visibly influenced, the spindle showing damages, the chromosomes showing incipient stickiness. The equatorial plates exhibit tendencies to melt down. Anaphases going on at the start of the treatment have been stopped in a pycnotic condition (Fig. 1 *y*). After 15 minutes decidely more serious damage is found. The resting nuclei of some roots have already lost most of their stainability; their shape is often spinous and distorted (Fig. 1 *a*). High up in the roots the nuclei already show full alkaline swelling. Metaphases and anaphases are strongly pycnotic (Fig. 1 *x*). 30 minutes' treatment brings about far-reaching changes. In the meristem the cells show signs of maceration, beginning to fall apart from each other. Their nuclei are highly swollen and the chromatin forms many irregular bodies (Fig. 1 *h*). In the older parts of the periblem the first indications of despiralisation may now be found. 1 hour's treatment gives tendencies to despiralisation, also to meristematic cells; but hardly until after 2 hours is it possible to find the first cases of full despiralisation of metaphase chromosomes. This latter condition is rather rare, however, because at that time most of the stainable material has disappeared, which may mean that the chromosomes have dissolved. Therefore, no full despiralisation can be found at the sequent time of fixation, after 8 hours.

In lower concentrations, 0,075 and 0,050, broadly speaking the same behaviour as now described for 0,1, is met with. The lethal changes, however, take place a little more slowly. After 30 minutes the poisoning is already pronounced in 0,075, and the first signs of despiralisation are to be seen after 1 hour. Now also 0,050 starts giving visible toxic effects, and after 4 hours 0,075 has reached full despiralisation and 0,050 incipient despiralisation. In 0,050, and lower concentrations down to 0,030, full despiralisation is present during the time interval 12—24 hours after the beginning of the treatment. Since the dissolution of the matrix evidently occurs at a slower rate in these concentrations, full despiralisation lasts for at least 12 hours, while it passes in about 1 hour in the concentration 0,1.

0,025 marks the lower limit of the typical alkaline swelling of the nuclei. Still after 2 hours' treatment no clear signs of toxic action are visible, but after 4 hours all metaphases have fallen together into pycnotic lumps in the centre of the cells. The resting nuclei are coarser in structure. 12 hours' treatment gives more pronounced changes: the resting nuclei are swollen, and the mitotic chromosomes are elongated. No total swelling-up of the resting nuclei and no total despiralisation of metaphase chromosomes are found in this concentration even after very long treatments.

It should be mentioned that YAMAHA (*l. c.*) reports both the swelling of nuclei and the dissolution of the chromatin in the strongest KCN solutions used by him.

C-MITOTIC ZONE (0,01—0,000·25).

C-mitosis is the collective term for those mitotic deviations effected by the partial or total destruction or inactivation of the spindle function. In the case of KCN we regard 0,01 as the upper limit of this reaction, although the spindle certainly is destroyed also within the lethal zone. 0,01 is, however, the first concentration in which the cells live long enough to exhibit any vital reactions. The c-mitotic zone may be divided into 2 sub-zones: an upper one, 0,01—0,002·5 in which stickiness greatly overshadows the c-mitosis, and a lower one, 0,001—0,000·25 in which c-mitosis may be just as typical as in colchicine or in any of the narcotic inducers of c-mitosis.

The upper c-mitotic zone (0,01—0,002·5) induces changes strikingly dissimilar to those of the lethal zone. In Fig. 1 *q*, *r*, two instances of nuclei are drawn from this treatment zone, and they are to be com-

pared with the nuclei of the upper part of the same Fig. The alkaline swelling of the nuclei is missing; and they are small, condensed and intensely stainable. Evidently the difference in pH between 10 of 0,025 and 9,5 of 0,01 is decisive for this condition. The structure of the nuclei after 12 hours in 0,01 is rather pycnotic. The metaphases show all transitions from relatively undamaged, although somewhat sticky, metaphases with the chromosomes well in order on the spindle (Fig. 1 *s*), some with their arrangement upset (Fig. 1 *t*), highly pycnotic chromosomes with lampbrush structure (Fig. 1 *u*), to those showing »star-metaphase» (Fig. 1 *v*) or »ball-metaphase» (Fig. 1 *w*).

The occurrence of »normal» metaphases or anaphases after 12 hours' treatment in a concentration, in which certainly the spindle apparatus has been immediately inactivated, is worthy of observation. The presence of apparently normal mitoses is no guarantee for a normal spindle function. It may be that such changes of the physiology of the cell have been induced by the treatment that any chromosome movements are arrested, a condition which will tend to preserve the chromosome arrangement as it was at the start of the treatment. The less any general toxic effects are associated with the c-mitotic action of a substance, the sooner will the chromosomes assume c-mitotic type and arrangement.

As already mentioned, the c-mitosis of the upper c-mitotic zone is characterized by a pronounced stickiness, which confers a pycnotic type to the c-pairs. Consequently the chromatids of each c-pair stick together; the characteristic »diakinesis»-type of c-pair, 8-like and cruciform ones, do not occur. Neither do we find any c-anaphases with their »ski-pairs». Even if metaphase were able to proceed into anaphase, the stickiness would check the falling apart of the anaphase chromosomes. Usually the c-mitosis of these concentrations will never enter any normal c-telophase; instead, most of them will end as irregularly shaped pycnotic lumps of chromatin.

The degree of contraction of the c-pairs is abnormally small, which constitutes another abnormal feature of the c-mitosis of this concentration zone. Ordinarily, a strong chromosome contraction goes hand in hand with c-mitosis. This c-contraction evidently cannot express itself in the presence of the strong toxicity of these concentrations.

The lower c-mitotic zone (0,001—0,000.25) is not at all free from general toxic effects. These, however, do not check the cells from exhibiting a fairly normal and complete c-mitosis. The c-pairs of Fig. 2 *p*—*w* (except *u*) are representative of this zone. It will be seen that

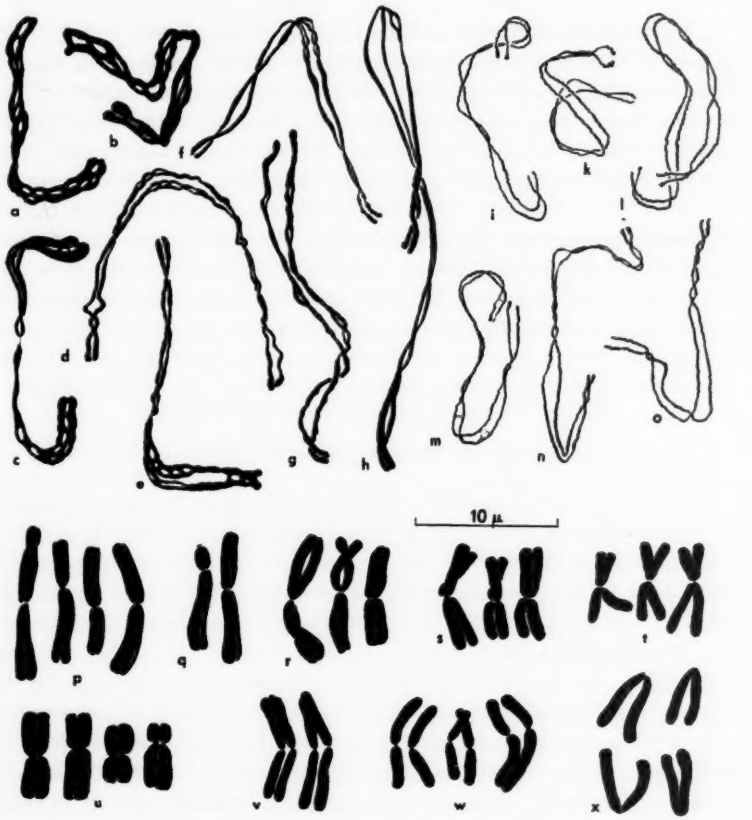

Fig. 2. Single chromosomes. *a*—*o*: from the lethal concentration zone, *p*—*t*, *v*—*w*: from the c-mitotic zone, *u*, *x*: from below the c-tumour zone. *a*—*c*, *d*—*h*, *i*—*o*: chromosomes from three cells showing various stages of despiralisation; *p*—*w*: various appearances of c-pairs, *x*: normal anaphase chromosomes. Dosages: 0,075 mol/l, 30 min: *a*—*h*; 0,05 mol/l, 12 h: *i*—*o*; 0,005 mol/l, 12 h: *r*; 0,001 mol/l, 12 h: *p*; 0,000'5 mol/l, 12 h: *q*—*v*; 0,000'25 mol/l, 18 h: *s*, *t*, *w*; 0,000'01 mol/l, 6 days: *u*, *x*. — ×2200.

even here the chromosomes are somewhat sticky and somewhat failing in contraction. They are most typical in the weakest concentration of the zone, 0,000'25, where they may show 8-shape (Fig. 2 *r*), cross-shape (Fig. 2 *t*), as well as typical ski-pair-shape (Fig. 2 *w*). Certain roots of this treatment start revealing signs of spindle action, as formation of multipolar spindles; it is evident that this concentration is close to the lower limit for the c-mitosis reaction.

A prolonged treatment within this zone will give various results in various concentrations: 0,001 and 0,000·5 exhibit pronounced signs of poisoning after 2 $^1/_2$ days of treatment. Usually the roots are losing their turgor at that time. The resting nuclei have lost their structure, they are homogeneously and faintly stained. Division stages may be found strongly distorted in their c-mitotic orientations, often developing into pycnotic ball-metaphases. Occasionally 0,000·5 has allowed the mitoses to rescue themselves into resting stage, in which case no pycnotic divisions are seen in the tissue. This latter condition is the rule with 0,000·25, which after 2 days contains only resting nuclei of normal appearance. This concentration allows one c-mitotic cycle to reach its conclusion and enter normal c-telophase. The occurrence later on of tetraploid mitoses has been observed and is in accordance with this fact.

C-TUMOUR ZONE (0,000·1—0,000·025).

The zone immediately below the c-mitotic zone is characterized, as already mentioned earlier, by the formation of c-tumours. Also here it must be pointed out that the upper limit of this zone is determined by the toxicity of the solutions, which do not permit any root growth and therefore suppress any possibility of such a reaction as the c-tumour growth to manifest itself.

Within this zone the spindle is functioning normally. In the strongest concentration, 0,000·1, however, a remarkably strong chromosome contraction was repeatedly noticed. It is conceivable that the threshold for the c-contraction is situated somewhat lower than the c-mitotic threshold. This situation was demonstrated by ÖSTERGREN (1944) to be present in many cases.

Treatment during a long time with concentrations of this zone has shown the occurrence of c-mitotic tendencies, which occasionally may lead to typical c-mitosis. Thus, we found that 0,000 1 and 0,000·05, after having given exclusively normal mitoses during the first few days of treatment, on day 6 unexpectedly started inducing clearcut c-mitoses in some roots. Since, however, most roots had only normal mitoses, this late type of c-mitosis is interpreted by us as belonging to the type of c-mitosis now and then occurring as a consequence of certain influences. If a root meristem, for instance, gets killed in one way or another, it happens that mitoses of cells in the neighbourhood of the dead sector take on a c-mitotic appearance or even enter full c-mitosis. It seems that through the long treatment in weak KCN solution a toxic

effect may accumulate in certain roots, which eventually leads to mitotic disturbances of the c-mitotic type. Even as far down as in 0,000'01 there occurred one instance of this on the 6th day of treatment. Three roots were healthy and contained exclusively normal mitoses (Fig. 2 *x*): one root, however, was of an unhealthy type, and in this one there occurred c-mitoses of a rather pronounced type with a high degree of c-contraction of the c-pairs (Fig. 2 *u*).

As already mentioned, the c-tumours go as far down as 0,000'01. Thus, this reaction has 25 times lower threshold value in KCN than the c-mitosis reaction. Only below the concentration of 0,000'01 we find quite normal conditions without any morphologically visible disturbances of cellular life.

THE DESPIRALISATION REACTION.

Among the various reactions, which have been briefly analysed on the preceding pages, the despiralisation phenomenon calls for special attention. Great interest is centered in it, mainly because of the information it may yield about the organisation of the contraction stages of the chromosomes.

It has long been known, above all through the work of Japanese cytologists, that various agents may bring about an artificial unravelling of the chromonema. Among chemical agents alkaline solutions are known to lead to this end. Thus, OURA (1936) succeeded in despiralising the major coil of the bivalents of *Tradescantia* by treating the pollen mother cells for a few minutes with 0,06—0,002 KCN. Later on, the same author in KUWADA, SHINKE and OURA (1938) added to the material of chromosome despiralisation by KCN by unravelling heteropycnotic chromosomes or chromosome parts during resting stages, as well as various small metaphase bivalents. The dosage employed in these latter cases was 0,008—0,004 KCN for 3—30 minutes. The effect obtained is interpreted as a dissolution of the matrix substance of the chromosomes effected by the alkaline reaction induced to the cell by the treatment.

As mentioned above, RYLAND (1948) studied the KCN-effect on *Allium* root tips in the dosages 0,05—0,001 for 25 hours. She observed a differential effect of the poison in different tissues of the root. In the strongest treatment she describes the presence of unusually extended »ragged» chromosomes with lampbrush appearance. Matrix had to a great extent disappeared. The microphotos Nos. 27, 28 and 30 in her paper clearly show despiralised chromosomes.

Our series 3 and 5 were arranged for illuminating the conditions of the despiralisation effect, as well as its dependence on concentration and time of exposure. Is the despiralisation phenomenon absolutely restricted to a narrow concentration zone? How far is it possible to make up for a decrease in concentration by an extension of the exposure time?

It turned out that a more banal type of despiralisation may be distinguished from what is here called total metaphase despiralisation.

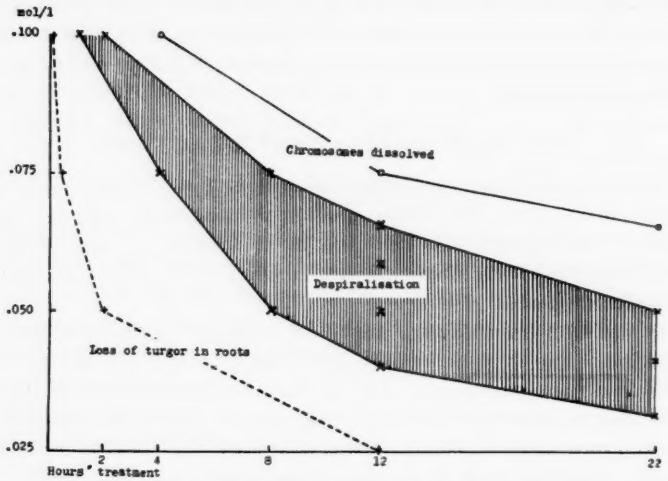

Fig. 3. Graph of the development of the despiralisation reaction.

This banal type is a reaction common to a great many treatments with various substances, while the total despiralisation has rather restricted limits for its realization. In Fig. 3 a graph is given summarizing the conditions for the appearance of the total despiralisation as observed in our series 3 and 5. Within the lined area of the graph we observed total metaphase despiralisation in the dosages marked out by crosses. Thus we found that it does not occur below 0,033. The next concentration 0,025 was never able to induce the complete reaction even after a long period of treatment. The despiralisation appeared only after the cells had died, and a series of characteristic changes had taken place in the cells. Thus the turgor of the roots had vanished long before the onset of the despiralisation (see the lowest curve of the graph).

As already briefly sketched above, the introductory changes to the

despiralisation consist of the development of strongly pycnotic conditions in resting nuclei and in division stages. The next step is the swelling up of the resting nuclei, which comes at about the same time as the first signs of despiralisation. Usually the despiralisation is first seen in prophase chromosomes, which may be connected with their being less charged with matrix substance. The metaphase chromosomes exhibit a pronounced stickiness at the same time as the chromosomes

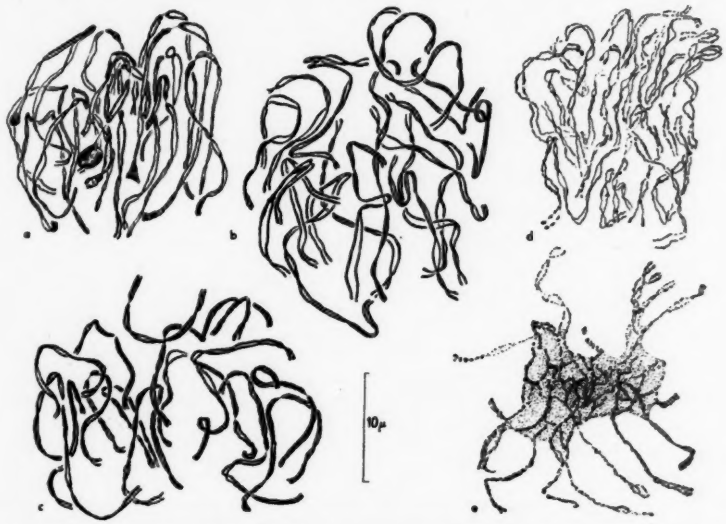

Fig. 4. The appearance of despiralised chromosomes *in situ*. *a*: prophase, *b*—*e*: prometaphases or full metaphases. Dosages: 0,058 mol/l, 12 h: *a*; 0,041 mol/l, 22 h: *d*; 0,033 mol/l, 12 h: *c*, 22 h: *b*, *e*. — ×1650.

are strongly elongated. Fig. 2 *a*—*c* and *d*—*h* are instances of such chromosomes drawn from two cells treated with 0,075 for 30 minutes. The matrix substance is flowing along the chromosome surface, and the chromosomes show holes at various places, sometimes indicating a subdivision of the chromatids (for instance, Fig. 2 *d*—*g*).

Gradually the chromosomes get thinner (Fig. 2 *i*—*o*), while lampbrush structures indicate that chromatin goes out from the chromosomes. So far the despiralisation follows a similar pattern in KCN as in other alkaline solutions, NaF, phosphate buffers, *etc*. With KCN, on the other hand, there is in addition a further-going, more extreme despiralisation, which, even if it is not entirely specific for this substance,

is only seldom met with in other treatments. Evidently, especially fortunate circumstances are necessary in order to direct the dissolution of the chromosomes in such a manner as to deprive them of most of their matrix substance without simultaneously damaging the internal structure of the chromosome thread beyond recognition.

In Figs. 4 and 5 instances are given of the appearance of the KCN despiralisation. A very common deviation from the complete reaction, or maybe a preliminary stage of it, is represented by Fig. 4 *e*. Here

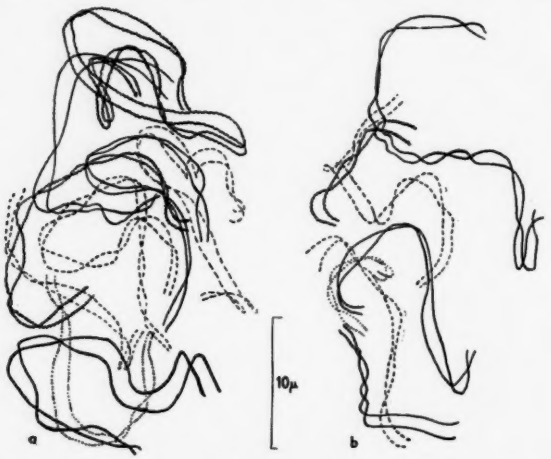

Fig. 5. The chromosomes of one totally despiralised metaphase or prometaphase schematically drawn *in situ*. — ×1900.

evidently the central part of the metaphase plate has become too crowded to allow an even dissolution of the matrix substance from the chromosomes. Therefore, the entire central part of the plate forms one irregular chromatic cluster, out of which certain despiralised chromosome arms are stretched. For a total despiralisation it would seem necessary that the chromosomes lie relatively free from each other allowing the matrix to be attacked equally from all sides. This may be one cause why prophase despiralisation is more easily attained than metaphase despiralisation.

Fig. 4 *a* shows a despiralised prophase with pronounced polarization of the chromosomes. A nucleolus is still attached to the satellited chromosome. The centromeric region may be recognized in many chromosomes as a rather wide unstained gap, on both sides enclosed by

chromatic material, somewhat more deeply stained than the rest of the chromosomes. Many of the chromosome ends also stand out by reason of their deeper colour.

Fig. 4 *b* and *c* are two metaphases or prometaphases, where the despiralisation has gone far without being maximal. Their longest chromosomes are 30—35 μ. They have the typical appearance: their chromatids being widely separated but running parallel. Especially in *c*, the centromeres are discernible. The chromatids are involved in a relational spiral, which often is at its densest towards the ends. Since this relational spiral often forms decidedly more loops than are present in ordinary metaphase or prometaphase chromosomes, there is no doubt that its development is due to the unravelling of the internal spiral of each chromatid. The individual chromatid spirals are transformed, by rotation of the chromosome arms, into a common relational spiral, which later on gradually disappears. Thus, the KCN elongation of the chromosomes undoubtedly involves a moment of real despiralisation, although much of it may be due to other factors such as a swelling and loosening of the chromosome structure.

In Fig. 4 *d* a very far advanced stage of despiralisation is drawn, in which the internal organisation of the chromosomes is beginning to disintegrate or in which parts of the chromosomes at least have lost their capacity of being stained.

Fig. 5 is an instance of how a highly despiralised cell, probably at metaphase, has still kept its chromosomes sufficiently undamaged to permit a complete analysis of all of them. The picture is drawn somewhat schematically in order to make it possible to follow the course of all chromosomes in their entire length. The general appearance of this cell may be seen from the photomicrographs in LEVAN, 1949 (Fig. 2 *k*—*l*). According to our opinion, there is no doubt that a suitable despiralisation by KCN may be of use even in the routine chromosome morphological analysis, and also by adding new aspects to the study of the internal chromosome mechanics. The two chromatids of each chromosome of Fig. 5 show a very delicate structure. They are built up from thin, almost unstained fibres and chromomeres, which often are of a characteristic size and appearance on the same level in both sister chromatids. Approximately the expected relations in chromosome length are found, the longest chromosome being about 60 μ in length, the shortest one about half that length. This means that the elongation in relation to ordinary metaphase chromosomes is about 5—6 times, and compared with maximally c-contracted chromosomes about 10—12

times. As seen from this picture, and also from Fig. 4 *b* and *c*, the chromosome arms often turn around in a manner suggestive of a relic coiling. We do not know whether this is due to a relic coiling already existing in the chromosomes at the beginning of the treatment or whether the unravelling of the chromatid coil may cause these big curvatures of the chromosomes.

SUMMARY.

Allium tests with KCN revealed the following effects on nuclei and chromosomes in the following concentration zones:

(1) *Lethal zone* (from 0,025 upwards). A strong alkali dissolution of chromatic material is found, eventually leading to the complete washing out of all stainable material from the cells. Resting nuclei show a very characteristic swelling-up. Division stages get pycnotic and gradually develop elongation of the chromosomes. Maximal despiralisation is attained under rather particular conditions. The dosages giving full despiralisation of metaphase chromosomes are represented in the graph of Fig. 3 by the lined area.

(2) *C-mitotic zone* (0,01—0,000·25). This zone may be subdivided at 0,002·5 into one upper part, where the c-mitoses are highly influenced by the general toxicity of the treatments, mainly a pronounced stickiness, and one lower part, where c-mitosis, at least during the initial period, takes a more or less normal course resulting in normal tetraploid c-telophase nuclei.

(3) *C-tumour zone* (0,000·1—0,000·025). Here three days of treatment induce typical, although not very large, c-tumours. Within this zone mitoses are normal. A prolonged treatment (6 days) may start c-mitotic tendencies even in the concentrations of this zone, and occasionally full c-mitosis. This c-mitosis is thought to have another mechanism of induction than the initial c-mitosis of the c-mitotic concentration zone. Below 0,000·025 no visible disturbances of cellular life were met with, and from 0,000·01 and downwards root elongation goes on normally.

In the final chapter an analysis is given of the KCN-induced despiralisation phenomenon.

May, 1952.

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CHROMOSOMES IN GERANIUM BOHEMICUM AND G. LANUGINOSUM WITH SOME NOTES ON THE HYBRID

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IN other papers the author has treated of the two nearly related species *Geranium bohemicum* L. and *G. lanuginosum* LAM. and their interesting oecology. The seeds preserve their power of germination during periods of human generations, but the seeds generally do not germinate without being exposed to heat. Consequently, these species often appear suddenly after forest-fires or in places where rubbish has been burnt, but also on recently cleared soil heated by insolation (*cf.* also DAHLGREN, 1948). From a phytogeographical respect it is noteworthy that *G. lanuginosum*, which for a long time has been regarded as a Mediterranean plant, has also been discovered in different places in Sweden. (Dr. H. STENAR, Södertälje, has in a letter told me a new locality for *G. lanuginosum*: Småland, Överum, $^4/_9$ 1943, where the rare plant was collected by Mr. E. MALMSTRÖM.)

To determine the number of chromosomes of the two species has met with peculiar difficulties. For some reason the fixation of root tips has not always turned out especially well, and it was rather striking that in some cases the nuclear divisions were also quite rare. It must moreover be added that the chromosomes would often be rather difficult to distinguish from each other by counting. However, after many years of repeated fixations, the author has finally succeeded in getting a small collection of good slides, particularly after abandoning staining with ferric haematoxylin and turning to gentian violet. WARBURG (1938, p. 132) also holds that staining with haematoxylin has »proved quite useless for the Geraniaceae». As fixative MÜNTZING's (1933) modification of NAVASHIN's fluid has been used.

The lists of chromosomes in DARLINGTON and JANAKI AMMAL (1945), A. and DORIS LÖVE (1948) and TISCHLER (1950) include »cultivated» plants (also such as occur in botanical gardens only), the North-European species and finally those of Central Europe. Quite recently, Madame CÉCILE DELAY (1951) has also published a more extensive list

of the known numbers. With particular reference to the work of WARBURG on taxonomy and chromosome number in the Geraniales she has given a table of the *Geranium* species investigated. GAUGER's (1937) collocation, however, is not cited. The chromosome numbers are now known in nearly fifty species of this genus.

G. bohemicum. — As we know, a plant species sometimes comprehends types with different numbers of chromosomes. The author has therefore investigated some plants, raised from seeds that were harvested from specimens originating from places widely separated within the distribution area of the two species in Sweden (maps by DAHLGREN, 1943). The seeds were treated with hot water and, if swelling, sown in pots. It turned out that all the individuals of *G. bohemicum* that were examined had $2n=28$ (Fig. 1 *a*). They came from the following localities: S w e d e n. *Östergötland*: Skedvi, Byle. *Södermanland*: Gryt, Rösunda; Tyresö. *Uppland*: Blidö. *Dalarna*: Hedemora, Granby. *Medelpad*: Timrå. — N o r w a y. *Oslo.* (Two individuals, which were descendants of a plant that had grown up from an 80-year-old seed.)

Our two *Geranium* species belong to the section Columbina, which (together with the section Robertiana) comprehends the annual plants. These are remarkable because of their rather aneuploid circumstances with $2n=18$, 22, 26, 34, 42, and 46 or 48. The chromosome number 28 in *G. bohemicum* is not only new to the section but also noteworthy as the most common one in the genus as a whole. The original basic number is therefore probably 14. *G. sanguineum* has the highest number of chromosomes, $n=42$, $2n=84$. With the basic number 14 this species must be regarded as a hexaploid.

G. lanuginosum. — As regards our second species, *G. lanuginosum*, WARBURG (using the synonym *deprehensum* ALMQ.) has reported (1938, p. 142) that the diploid number here should be 42. In comparison with the 28 chromosomes in the so closely related *G. bohemicum*, this is almost identical with saying that in *G. lanuginosum* we have a case of triploidy. The fertility, however, in the last species is quite satisfactory. In another section WARBURG has hit upon a typical triploid, *G. platypetalum*, which is only vegetatively propagated in gardens. On account of meiotic disturbances (*l. c.*, p. 148) no pollen grains are developed.

A re-investigation of *G. lanuginosum* was, of course, highly desirable; and that all the more so because the species in question has at times been confused with the habitually rather similar *G. bohemicum*. At the examination of our slides it has now appeared that the $2n$-

number in *G. lanuginosum* must be 48 (Fig. 1 *b*). This was first proved on a plant of unknown provenience and later also in individuals from the following three localities: *Småland*: Lofta, Ekvik. *Södermanland*: Tyresö (DAHLGREN, 1949, p. 363). *Dalsland*: Gunnarsnäs, Rostock.

Perhaps the number 48 is not quite unique among the annual species of the Columbina section. WARBURG has stated that he has seen 46 or 48 chromosomes in *G. carolinianum*. The number of chromosomes in this section are, we repeat, very varying.

But how are the counting and drawing of 42 chromosomes in *G. lanuginosum* to be explained? Probably the whole discrepance is

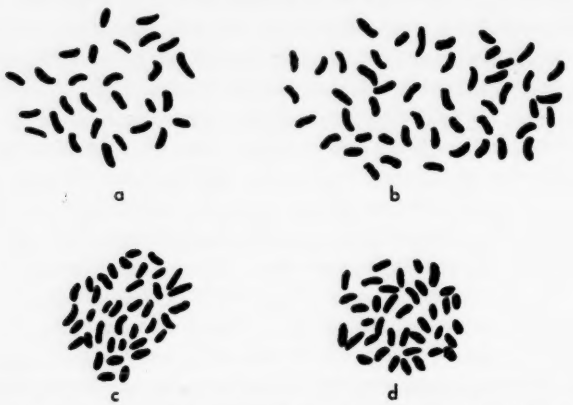

Fig. 1. Somatic chromosomes from *a*: root tip in *Geranium bohemicum*, 28; *b*: root tip in *G. lanuginosum*, 48; *c* and *d*: ovula in the hybrid *G. lanuginosum*×*bohemicum*, 38 (=24+14). — Magnification 1900.

simply due to a mistake. As was said before *G. lanuginosum* has been a rather troublesome object. *A priori*, however, it is not quite impossible that a type could exist with 42 chromosomes. If WARBURG has confounded the two species and accidently met with an autotriploid plant of *G. bohemicum*, this would also have had 42 chromosomes. In root tips not only polyploidy has often been observed in cells but at times also other irregularities. Chromosome aberrations caused by extreme temperatures are known. Quite recently BRUHIN (1951, p. 565), for instance, has established that the achenes of *Crepis aurea*, which during a year have been exposed to varying temperatures in the Alps (during summer sometimes more than 50° C), could give rise to chromosomal changes, at times also total disappearance of whole chromosomes.

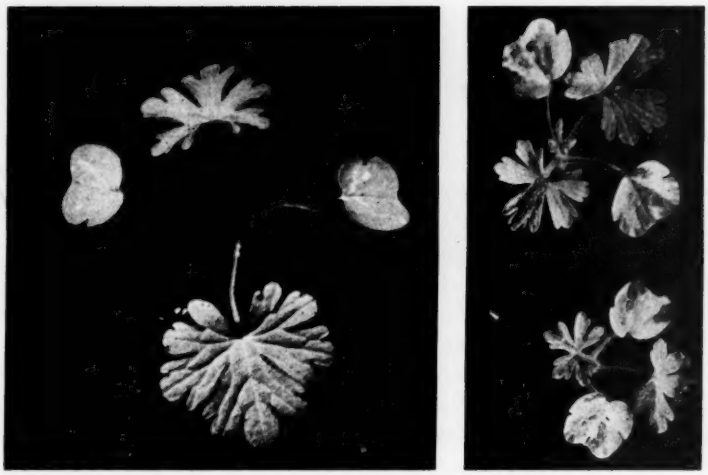

Fig. 2. To the left, a peculiar spontaneous seedling of *Geranium lanuginosum*×*bohemicum* only showing the normally dominating incision on one side of each cotyledon. The spotting of the cotyledons is less pronounced than in the reversed combination *G. bohemicum*×*lanuginosum* (to the right).

Strong heating of the seeds is often necessary for the germination of our two *Geranium* species. This circumstance might perhaps be conceivable as an inducement to bring about a reduced number of chromosomes in a root of WARBURG's material. But, as has already been said, the whole matter is probably due to a mistake in his observations.

G. lanuginosum×*bohemicum.* — The author (1923, 1925) has previously reported on hybrids between *G. bohemicum* and *G. lanuginosum.* It is peculiar that the F_1 plants, at least as seedlings, are more or less spotted in green and white, but this in a very varying degree, depending on which of the two species has been the mother plant (Fig. 2). In hybrid cells only the *lanuginosum* plastids have the power to develop into normal chloroplasts. The combination *G. bohemicum*× *lanuginosum* (the hybrid originates from an egg-cell without plastids capable of normal development) consequently gives a more pronounced white patching than the inverted combination, *G. lanuginosum*×*bohemicum,* in which the suitable plastids in the egg-cell are much more numerous than the plastids of the *bohemicum* type which have been sparsely transferred by the pollen tube. That really plasma via the pollen tube can be transferred into the egg-cell has lately been cytolog-

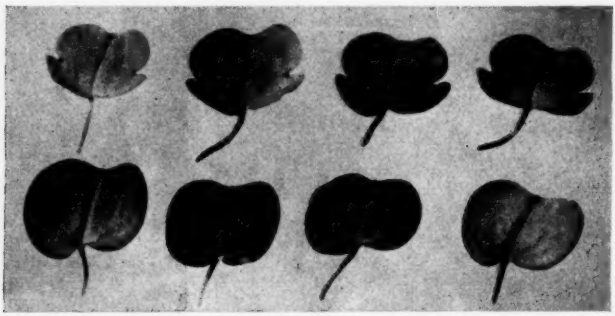

Fig. 3. Above cotyledons from *Geranium bohemicum* and below from *G. lanuginosum.*

ically demonstrated by STEFFEN (1951, p. 230) in *Impatiens glanduligera* (syn. *I. Roylei*). During the development of the hybrid plants a segregation of the two kinds of plastids occurs during the cell divisions, and so pure white, variegated and pure green leaves and shoots may appear (Fig. 2).

It may also be noted here that Mrs. SVERDRUP (1924) has made the crossing *Geranium striatum*×*Endressi* and got seedlings with white spotted cotyledons. She also found that the following young leaves were »showing a certain amount of variegation which disappeared almost entirely later on». The reversed combination, however, resulted in total green plants. Except for a short notice on the F_2 plants (1925) she did not publish anything about her experiments. [SANSOME (1936, p. 359) has touched upon this hybridization in connection with his studies on male sterility.] In his treatise on vegetative segregation CHITTENDEN (1927, p. 404), too, has reported that »such cases are under observation in hybrids between several species of *Geranium*». Chlorophyll defective descendants after species crossings have also been obtained in *Oenothera* (RENNER), and recently SMITH (1951) has established the same in species hybrids within the genus *Melilotus.*

The two species sometimes grow in the same burned place (DAHLGREN, 1945, p. 384; 1949, p. 363). Self-fertilization normally occurs, but cross pollinations are not excluded. On 8 Aug., 1951, the author observed in Uppsala Botanical Garden a bumble-bee, which, after first having visited six flowers on *G. bohemicum*, immediately afterwards sniffed at two others in a *G. lanuginosum* plant growing in a pot two metres away. Earlier the author has also reported on a *spontaneous*

hybrid between the two species (DAHLGREN, 1949), and in 1950 a new one appeared after the same combination, *G. lanuginosum*×*bohemicum*. The cotyledon type of *G. bohemicum* dominates (Fig. 3); for the rest the hybrid is quite like *G. lanuginosum* but totally sterile.

It was very surprising that the cotyledons in the hybrid of 1950 only possessed an incision at one side of each cotyledon (Fig. 3). Consequently, dominating and recessive characters from both parents were combined in the cotyledons.

Finally, it may be mentioned that the chromosome number (Fig. 1 *c* and *d*) of this curious hybrid could at last be determined in young ovules and, as anticipated, was 38 (=24+14).

The author is very grateful to Mrs. GURLI ANDERSSON, the skilful preparatrice of the institute, for her assiduous work on this difficult material. He also wishes to thank Lic. phil. S. BJÖRKMAN for his kindness in fixing roots not only in the darkness of the night but also quite early in the morning.

Summary. — (1) The somatic chromosome number in *Geranium bohemicum* is 28, a unique number in the section *Columbina*, but quite common among the perennial species. — (2) The nearly related species *G. lanuginosum* has $2n=48$. WARBURG has stated that this plant has $2n=42$, but this is probably owing to a mistake. — (3) In a spontaneous hybrid, *G. lanuginosum*×*bohemicum*, the chromosome number could be determined as 38 (=24+14). — (4) This hybrid possessed an incision only on one side of its cotyledons, thus demonstrating a case that showed simultaneous dominance and recessiveness.

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Corrigendum. — While looking for my older *Geranium* papers in the earlier volumes of »Hereditas» I found with consternation that in my »Kreuzungskleinigkeiten» (Hereditas, V; 1924) I have written *Dracocephalum thymiflorum* instead of *D. Moldavica*, a *lapsus calami* which I now wish to correct.

X-RAY INDUCED CHROMOSOME BREAKS IN DROSOPHILA MELANOGASTER

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I. INTRODUCTION.

IN a previous paper (LÜNING, 1952 a) it was shown that the rate of X-ray induced dominant lethals in spermatozoa varied with the time from irradiation to insemination. The rate was nearly constant in the first 5 days after treatment. Then there was a sharp increase at the 6th to the 7th day. This high rate remained until the 10th day. At the 11th day there was a very sharp decrease that continued up to the 20th day after treatment, when the rate was nearly the same as in non-irradiated males. The present paper deals with the causes of these different rates of dominant lethals. Some of the experiments have been published in an abstract (LÜNING, 1952 b). They are yet included and further discussed below.

II. MATERIAL AND METHODS.

In these experiments four stocks were used. Wild type males (Oregon) were mated to *y w sn* females and closed-X males were mated to ($\widehat{XXY}$) *y v f*; bw^{VA} /L^2 *l*. The latter stock was synthetized from two stocks kindly put at my disposal by Dr. LEWIS and Dr. VALENCIA. All cultures and stocks were kept in incubator at $25° \pm 1°$.

Irradiations were made at the Radiophysical Institute by using a Siemens Stabilivolt apparatus of 155 kV, 6 mA and 1 mm Al. The dose was about 960 r units given in 2 minutes or a multiple of this dose.

The males were irradiated at a definite age, mentioned in each case in the tables. They were mated to virgin females in vials, 2—5 females per vial according to the expected rate of dominant lethals, to produce about the same number of offspring per vial in all series. The males were transferred to new virgin females at certain intervals. These intervals were determined by the results briefly outlined in the introduction. Thus the intervals were the 1st—6th, the 7th—10th and the 11th—14th day after treatment. This partitioning is consequently a natural one.

The females were allowed to lay eggs only when they remained

with their mates. The vials were supplied with cubes of corn-meal agar that were changed every second or every third day. When changing the agar-cubes the flies were transferred to new clean vials. The old cubes with eggs and larvae were left in the old vials and supplied with tissue paper moistened in yeast-suspension. By this method the yield per vial is large and the flies reach maximum size. The spread in the emergence period is very small. 10 days after the end of egg-laying all flies had hatched (SPENCER, 1943).

III. THE PROBLEM.

Before studying the causes of the variation in the rates of dominant lethals, one ought to know the mechanisms at work in producing the dominant lethals. LEA and CATCHESIDE (1945) reviewed this subject supposing two different chromosomal causes.

Type I. According to MULLER (1940) and PONTECORVO (1942), dominant lethals are due to single breaks where the broken ends remain unjoined and then by sister union of chromatids they give a dicentric chromosome and an acentric fragment. The dicentric chromosome will be lost in the later cell divisions.

Type II. In spermatozoa with two or more breaks asymmetrical interchanges will lead to large deficiencies or to loss of whole chromosomes.

Dominant lethals due to Type I are predominant at lower doses. At higher doses dominant lethals due to Type II will increase more rapidly than the first power of the dose.

Among the spermatozoa with two or more breaks there would appear cases with two breaks in the X-chromosome, one near the distal end and the other near the proximal end. If there is an asymmetrical interchange one would get a very short monocentric chromosome, leaving the greater part of the X-chromosome as an acentric fragment. If the remaining part of the truncated X-chromosome is short enough, individuals with such a chromosome may be viable. As they have only one whole X-chromosome — derived from their mother — and a very short segment of another X-chromosome — derived from their father — they are phenotypically males, nearest equivalent to XO males. They are *hyperploid males*. They may also arise by three-break changes when the distal part of the X-chromosome is inserted in one of the autosomes.

By using suitable marker genes the hyperploid males could be

distinguished from normal males (XY). In the present experiments three marker genes were used, two of them in the distal part of the X-chromosome, *viz.* *y* (for yellow body) and *w* (for white eyes). The maternal X-chromosome contained the recessive alleles and the paternal, X-ray treated, X-chromosome contained the wild type alleles in these three loci. In the hyperploid males the small piece of the paternal X-chromosome contained y^+ or $y^+ w^+$ if the distal break was to the left or to the right of *w*-locus. If the length of the extra piece was of no significance one would get many more cases with $y^+ w^+$ than with only y^+ in the extra piece of the X-chromosome. In the material presented below there are 178 with only y^+ and 14 with $y^+ w^+$. Among the latter there were five cases with variegated eyes. It may be supposed that these five were due to breaks near and to the right of *w*-locus. The low rate of hyperploid males with a longer extra piece of the X-chromosome indicates that they were less viable. It is, consequently, a question of a difference of degree whether an aberration will cause lethality or appear as a hyperploid male. Hence, hyperploid males may be used to test if there are dominant lethals produced by the mechanism listed above as Type II.

BONNIER and LÜNING (1950) tentatively suggested that the high rate of dominant lethals in spermatozoa inseminated about 7 days after treatment was due to a change (decrease) in the rate of restitution of the broken ends. This means that the mechanism Type I is at work in increasing the rate of dominant lethals and it does so at the expense of Type II. LÜNING (1952 a) showed by using LEA and CATCHESIDE's (1945) data that all breaks must remain unjoined and that, consequently, all lethals are due to Type I in the period with the high rate. Hence, it is possible to test the hypothesis by BONNIER and LÜNING by using the occurrence of hyperploid males at different periods after treatment of the males.

IV. RESULTS.

In order to study the occurrence of hyperploid males from spermatozoa inseminated at different time after treatment, wild type males were irradiated at a definite age and mated to virgin *y w sn* females as is shown in Fig. 1.

The F_1 offspring consisted mainly of heterozygous females ($y\ w\ sn/y^+\ w^+\ sn^+$) phenotypically wild type and males phenotypically yellow white singed. There appeared some aberrant types. Among the females

there occurred some individuals, that were phenotypically yellow, white or singed. They are classed as »gene mutations» without any further analysis. Another type of the aberrations was the gynandromorphs. In these the paternal X-chromosome was lost in some part or parts of the body. In some gynandromorphs the whole X-chromosome was not eliminated. The male area was in these cases hyperploid. The third type

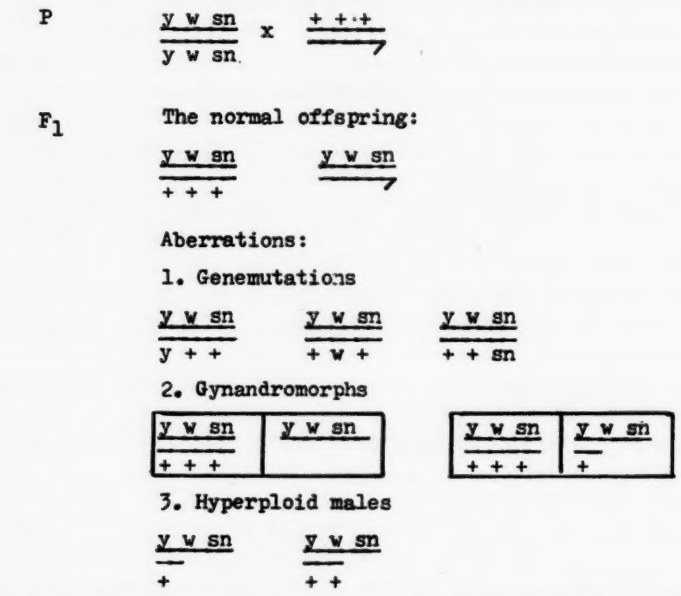
P
y w sn / y w sn
x
+ + + /
F1
The normal offspring:
y w sn / + + +
y w sn /
Aberrations:
1. Genemutations
y w sn / y + +
y w sn / + w +
y w sn / + + sn
2. Gynandromorphs
y w sn | y w sn / + + +
y w sn / + + + | y w sn / +
3. Hyperploid males
y w sn / +
y w sn / + +

Fig. 1. Genotypes of parents and offspring in Ser. 64, 65, 74, 75, 84, 96, and 97.

of aberrations listed was hyperploid males. The occurrence of these are described above. The constitution of the different aberrations is clear from Fig. 1.

In accordance with this plan the following experiments were carried out:

Ser. 64. 0—1 day old wild type males were irradiated with 960 r and immediately mated to virgin *y w sn* females.

» 65. The same males as in Ser. 64 were transferred to new virgin *y w sn* females at the beginning of the 7th day after treatment.

» 74. The same males as in Ser. 64 and 65 were transferred to new virgin *y w sn* females at the beginning of the 11th day after

treatment. They were discarded at the beginning of the 14th day after treatment.

Ser. 75. 6—7 days old wild type males were irradiated with 960 r and immediately mated to virgin *y w sn* females.

» 84. The same males as in Ser. 75 were transferred to new virgin *ywsn* females at the beginning of the 7th day after treatment. They were discarded at the beginning of the 11th day after treatment.

» 96. 0—1 day old wild type males were irradiated with 3840 r and immediately mated to virgin *y w sn* females. They were discarded at the beginning of the 7th day after treatment.

» 97. 6—7 days old wild type males were irradiated with 3840 r and immediately mated to virgin *y w sn* females. They were discarded at the beginning of the 7th day after treatment.

The results of these experiments are presented in Table 1. As hyperploid males were originally intended to be females, but had lost the greater part of the paternal X-chromosome their rates are calculated on the total number of females and gynandromorphs and not of normal (XY) males. In this table there is also included one column with the rates of dominant lethals corresponding to the different series, except Ser. 74. These percentages are taken from Table 2 but transferred from per cent of hatching to per cent of dominant lethals.

According to the hypothesis of BONNIER and LÜNING, referred to above, the rate of dominant lethals in Ser. 65 and 84 would mainly be due to Type I at the expense of Type II. In this hypothesis it is implied

TABLE 1. *Progeny from crosses of y w sn females to wild type males irradiated at an age of 0—1 or 6—7 days with 960 r or 3840 r. The males were transferred to new females at certain intervals.*

Series	Dose	Age of males at treatment, days	Period of insemination, days	Total females and gynandromorphs	*y, w, sn* mutations		Gynandromorphs		Hyperploid males		Dominant lethals in %
					Abs. nr.	per 10^4	Abs. nr.	per 10^4	Abs. nr.	per 10^4	
64	960	0—1	1—6	59856	8	1,3	35	5,8	12	2,0	20,79
65	960	0—1	7—10	62479	26	4,2	41	6,6	65	10,4	61,19
74	960	0—1	11—14	14165	0		1	0,7	1	0,7	
75	960	6—7	1—6	48336	18	3,7	20	4,1	14	2,9	28,90
84	960	6—7	7—10	39035	15	3,8	13	3,3	29	7,4	47,55
96	3840	0—1	1—6	21725	34	15,7	31	14,3	25	11,5	63,75
97	3840	6—7	1—6	24879	41	16,5	34	13,7	47	18,9	76,18

that the rate of breaks is the same in spermatozoa inseminated in the two periods 1—6 and 7—10 days after treatment. As hyperploid males indicate that mechanism Type II is at work in producing dominant lethals, one would expect a lower rate of hyperploid males in Ser. 65 and 84 than in Ser. 64 and 75. As is seen from Table 1, this is not the case. There is, instead, a sharp increase in the rate of hyperploid males in Ser. 65 and 84. Hence, the cause of variation in the rate of dominant lethals cannot be only a different rate of restitution of broken ends in the two periods. Another possibility of explaining the variation is to reject the implied hypothesis of a similar rate of breaks and suppose that it is *dissimilar*, and higher in spermatozoa inseminated 7—10 days after treatment than those ready the first 6 days. It is possible that there is an interaction between the two suggested causes, *viz.* higher rate of breaks and lower rate of restitution. In order to investigate this, Ser. 96 was planned. In this series 0—1 day old males were X-ray irradiated with a dose that would induce about the same rate of dominant lethals in spermatozoa, used the first 6 days after treatment, as in Ser. 65. It was supposed that a dose of $4 \times 960 = 3840$ r would do this. Comparing Ser. 65 and 96 in Table 1, one sees that both the rate of dominant lethals and that of hyperploid males is about the same in the two series. This indicates that the rate of dominant lethals due to Type II is about the same in Ser. 65 and 96. The simplest way to explain this is to suppose the same rates of breaks and restitution in spermatozoa used in Ser. 65 and 96. In Ser. 65 the dose was 960 r and in Ser. 96 3840 r. The effect of the treatment is, consequently, due to the stages that are treated.

The hypothesis about a differential breakability in different stages of the spermiogenesis will explain the results obtained by BONNIER and LÜNING (1950) and LÜNING (1952 a).

STRÖMNÆS (1949) as well as BONNIER and LÜNING (1950) demonstrated that the rate of dominant lethals varied also with the age of males at treatment. This statement is made on a comparison of data most closely corresponding to my period 1—6 days after treatment. First following this line we vill compare Ser. 64 *vs.* 75 and 96 *vs.* 97. In Ser. 75 and 97 the rates of dominant lethals and hyperploid males are higher than in the corresponding Ser. 64 and 96. The differences are greater between Ser. 96 and 97 than between Ser. 64 and 75 indicating that there is a dose dependence. To explain the higher rate of dominant lethals and hyperploid males in Ser. 97 than in Ser. 96, one may suppose a higher rate of breaks in spermatozoa used in Ser. 97

than in those used in Ser. 96. This means that a given dose will induce more breaks in spermatozoa used the first 6 days after treatment in 6—7 days old males than in 0—1 day old males. If this supposition is correct one would expect two different regression lines of the hatch-

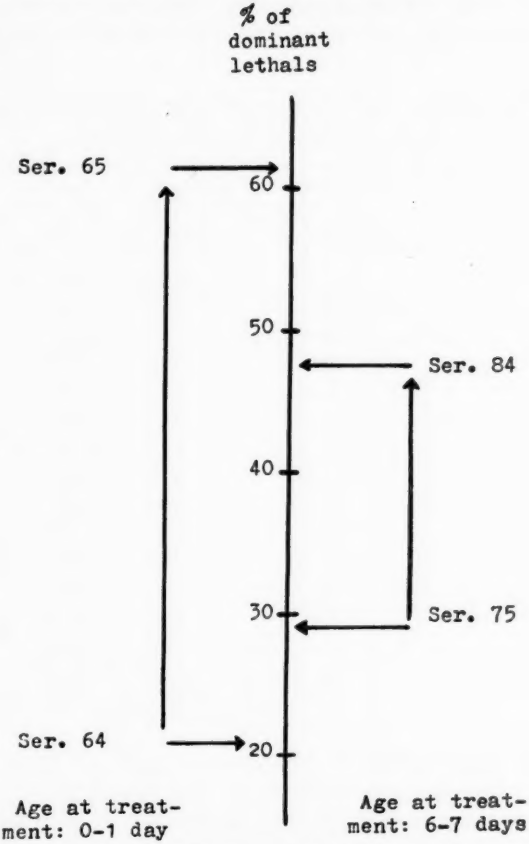

Fig. 2. Differences in the rates of dominant lethals in spermatozoa inseminated 1—6 and 7—10 days after treatment of 0—1 and 6—7 days old males with 960 r.

ability when treating 0—1 and 6—7 days old males with various doses. This is studied in the experiments presented in Table 2 and Fig. 3.

As there is a different breakability of chromosomes in spermatozoa used the first 6 days after treatment of males of different ages, *viz.* 0—1 and 6—7 days old, it is of great interest to study the effect on

spermatozoa used 7—10 days after treatment of the two groups of males. This will be done by comparison of Ser. 64 *vs.* 65 and 75 *vs.* 84. The differences are best demonstrated by a scheme (Fig. 2). On the scale the per cent of dominant lethals is set off. To the left the increase from Ser. 64 to 65 is marked and to the right that from Ser. 75 to 84. The latter is much less and this may be one of the causes why this effect

TABLE 2. *The relation between dose and hatchability in progenies from males irradiated at various ages.*

Dose	Age of males at treatment, days	Period of insemination, days	Total number of eggs	Percentage of eggs hatched
0	0—1	1—6	4299	88,30
	6—7	1—6	6467	87,54
960	0—1	1—6	5999	79,21
	6—7	1—6	5494	71,10
1920	0—1	1—6	5402	59,72
	6—7	1—6	3927	47,26
2880	0—1	1—6	4404	50,05
	1—2	1—6	5942	46,30
	2—3	1—6	4588	39,56
	3—4	1—6	2425	38,23
	6—7	1—6	3147	37,11
3840	0—1	1—6	6174	36,25
	6—7	1—6	6432	23,82
960	0—1	7—10	4370	38,81
	2—3	7—10	2880	43,37
	6—7	7—10	4723	52,45

has not been observed before. The increase from Ser. 75 to 84 may be explained in the same way as above for Ser. 64 and 65.

From the experiments presented in Table 1 we may now conclude that there is a different rate of breaks induced with the same dose due, on the one hand, to the age of the males at treatment and, on the other hand, to the time from treatment to insemination. This agrees with results of STRÖMNÆS (1949) and BONNIER and LÜNING (1950). According to the sensitivity — low to high — the order will be the following:

(1) 0—1 day old males irradiated. Spermatozoa inseminated 1—6 days after treatment. Ser. 64.

(2) 6—7 days old males irradiated. Spermatozoa inseminated 1—6 days after treatment. Ser. 75.

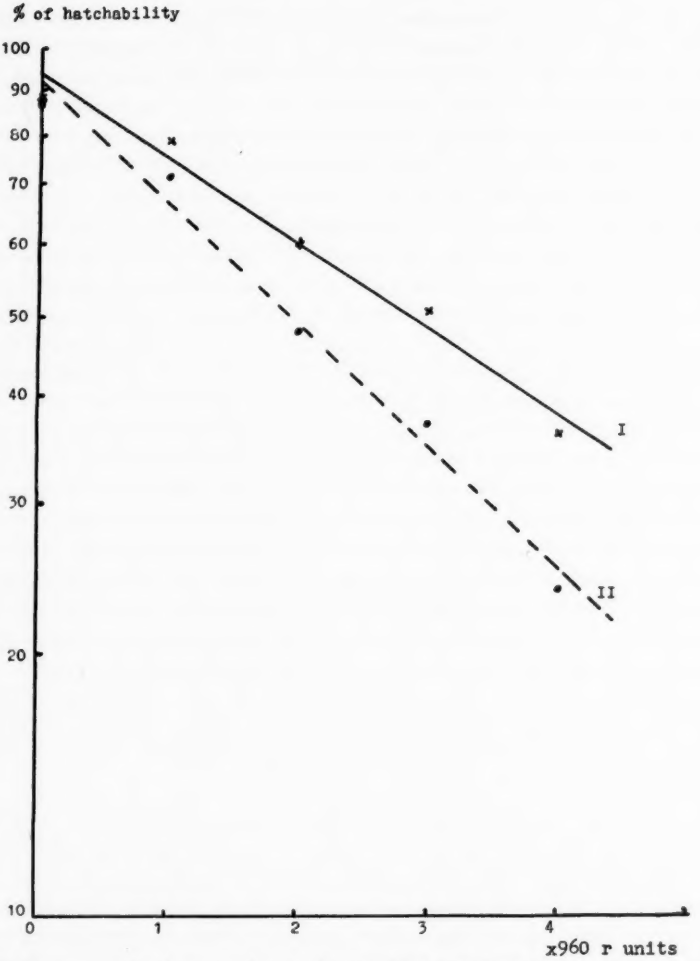

Fig. 3. The connection between dose and rate of hatchability when treating males of different ages, *viz.* 0—1 day (×=I) and 6—7 days (•=II).

(3) 6—7 days old males irradiated. Spermatozoa inseminated 7—10 days after treatment. Ser. 84.

(4) 0—1 day old males irradiated. Spermatozoa inseminated 7—10 days after treatment. Ser. 65.

Up to now only hyperploid males and dominant lethals have been discussed. In Table 1, however, two other types of aberrations are listed, *viz.* gene mutations and gynandromorphs. Primarily they were of secondary interest and were consequently not further analysed. The occurrence of gynandromorphs among the offspring after X-ray irradiation of males was reviewed by BONNIER, LÜNING and PERJE (1949). It was supposed that they were due to a break in a chromosome, after which splitting one of the chromatids restituted and the other remained open and was eliminated in a subsequent cell division, thus producing a mosaic with one part with female phenotype (XX) and another with male phenotype (XO). There also exist cases where the male area was hyperploid. (See Fig. 1.) As gynandromorphs are due to breaks, one would expect that the rates would be correlated to the rates of break. A comparison between Ser. 64 *vs.* 65 and 75 *vs.* 84 indicates that this is not the case. The variation in the rates of gynandromorphs lies within the random variation, $P=0{,}10$. Nor are there any considerable variations in the rates of gene mutations but a very low value in Ser. 64. Comparing Ser. 64 *vs.* 65 and 96 *vs.* 97, one sees that there is no difference between the latter. A repetition of Ser. 64 gave on a material of 51171 a rate of gene mutations of $2{,}54$ per 10^4. This, too, is lower than the results in Ser. 65, 75 and 84. Hence it is impossible to make any statement about a possible difference. A complete investigation of these small variations, if there are any, needs a much greater material than that presented here. A comparison between Ser. 64, 65, 75 and 84 *vs.* 96 and 97 shows that both the rates of gene mutations and that of gynandromorphs are dependent on the dose and seemingly not on the stages treated.

In Table 1 there is one series that has not yet been discussed. It is Ser. 74. LÜNING (1952 a) supposed that from the 11th day after treatment spermatozoa were used which were in a premeiotic stage at treatment. Ser. 74 was carried out to test this hypothesis. BONNIER and LÜNING (1951) showed that when females were X-ray irradiated there was no increase in the rate of gynandromorphs as compared to the control. It was supposed that this was due to the fact that when treating females it is not the female gametes that are treated but meiotic (metaphase I) or premeiotic stages. Breaks that are induced in this stage cannot cause an elimination that would lead to a gynandromorph. There

would be either a loss of a chromosome from the whole individual or no loss at all, but not a mosaic. By analogy with this it was supposed that if from the 11th day after treatment spermatozoa treated in a meiotic or a premeiotic stage were used, one would not get any gynandromorphs in Ser. 74. The result is in accordance with the expectation, thus supporting the hypothesis. The opinion that it would take about 10 days from meiosis until the spermatozoa were ready for insemination, was expressed by PONTECORVO (1944) from an analogism. This is confirmed by the present investigation.

It was supposed above that when treating both 0—1 day old and 6—7 days old males with various doses one would get different regression lines of the hatchability for the two ages.

In order to test this and also the time when the differences between 0—1 and 6—7 days old males appeared, the following experiments were made.

Groups of 0—1 and 6—7 days old wild type males were treated with 960, 2×960, 3×960 and 4×960 r. There were also two control series. The males were mated singly to virgin *y w sn* females in vials. Eggs were collected for counts from the 2nd to the 5th day after treatment. 1—2, 2—3 and 3—4 days old males were also treated with 3×960 r to enable the changes in the rates of dominant lethals with increasing age of males at treatment to be followed. According to STRÖMNÆS (1949), this would occur in males about 2 days old. 2—3 days old males were also treated with the dose 960 r. They were mated in the usual manner but eggs were not collected within the first 6 days after treatment. In the three series given 960 r, the males were transferred to new virgin females at the beginning of the 7th day after treatment. In these series eggs were collected from the 7th to the 11th day after treatment. All results are presented in Table 2.

The first task was to study if the difference observed above between 0—1 and 6—7 days old males was dependent on dose. In Fig. 3 the data from Table 2 for 0—1 and 6—7 days old males are set off, and the regression lines calculated. An analysis of the differences between the two regression-coefficients gives $0{,}02 > P > 0{,}01$. Hence, this experiment does not contradict the explanation of the differences between 0—1 and 6—7 days old males put forward above.

From Table 2 we may further infer that the increase in sensitivity appears in the group 1—2 days old males at treatment and that 2—3 days old males are only slightly different from 6—7 days old males.

It was of great interest to study whether the change in sensitivity

of spermatozoa inseminated 7—10 days after treatment of 0—1 and 6—7 days old males appeared at the same age of males as in spermatozoa

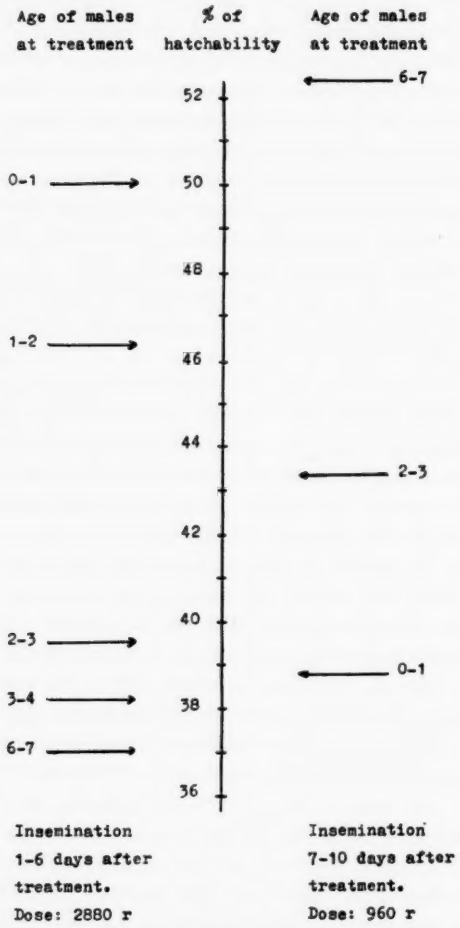

Fig. 4. Changes in the rates of hatchability of eggs fertilized by spermatozoa inseminated 1—6 and 7—10 days after treatment when varying the age of males at treatment.

used the first 6 days after treatment. To do this the three series in the bottom of Table 2 were executed. In this case the change was not completed in 2—3 days old males. The differences between the changes

in the two periods 1—6 and 7—10 days after treatment are clear from a scheme (Fig. 4).

MULLER (1940) presented a method of testing the rate of viable losses of X- and Y-chromosomes using a dominant allele of brown eye (bw^{VA}) in one of the second chromosomes and vermilion (v) in the attached-X chromosomes. Flies homozygous or hemizygous for v and heterozygous for bw^{VA} have eyes that are almost typically vermilion if an extra Y-chromosome is present, as in XXY females and XYY males, but their eyes are light variegated if there is no extra Y-chromosome present, as in XX females and XY males. With this method MULLER demonstrated that the frequency-dosage relation for losses of whole chromosomes was linear and concluded that it was due to single breaks that remain unjoined or with sister-union of the chromatids. At present it is suggested that variation of the doses is not the only method of varying the rates of induced breaks but that chromosomes in presumptive spermatozoa treated in various stages of spermiogenesis have a different breakability. Hence, one would expect the same effect with different doses on spermatozoa inseminated a limited time after treatment (MULLER) as with spermatozoa inseminated in different periods after treatment, according to the hypothesis of differential breakability presented above.

According to BAUER (1939, 1942) and CATCHESIDE and LEA (1945), the rate of losses of X-chromosomes is much higher when irradiating closed-X than when irradiating rod X-chromosomes. This is due to the fact that, when a single break in a rod X-chromosome restitutes, it may do so either in the original order or as a sister chromatid crossing over, both giving normal rod-X; but a single break in a closed-X gives two normal closed-X-chromosomes at the next division only when the chromatids restitute in the original order. When there is a sister chromatid crossing over one dicentric chromosome is produced at the next division with the subsequent elimination.

In the experiments carried out to test the hypothesis of a differential breakability of chromosomes in various stages of spermiogenesis MULLER's method in conjunction with use of closed-X-chromosomes was employed. The females used were $(\widehat{XXY})$ $y\ v\ f$; $bw^{VA}/L^2\ l$ and the males were taken from an X^{c^2} stock.

Ser. 127. 0—1 day old closed-X males were treated with 960 r. They were immediately mated to virgin females.

» 128. The same males as in Ser. 127 were transferred to new virgin

TABLE 3. *X- and Y-chromosome losses induced in various stages of spermiogenesis in 0—1 day old X^{c2} males.*

Series	Total number non-Lobe females	Variegated females	% variegated females
127	2676	20	0,75
128	1230	26	2,11

females at the beginning of the 7th day after treatment. They were discarded at the 11th day after treatment.

The results are presented in Table 3.

Comparing ser. 127 *vs.* 128, one sees that the rate of eliminations is about 3 times more frequent in Ser. 128 than in Ser. 127. This is an underestimate because a constant is included corresponding to the spontaneous rate of elimination (by MULLER it was 0,2 %). Subtracting this from both percentages, one gets a proportion that is about 3,5. This means that there are induced about 3,5 more breaks in spermatozoa inseminated 7—10 days after treatment than in those used the first 6 days. Comparing this with the results from Ser. 65 *vs.* 96 where the latter were given a dose 4 times that in Ser. 65, one sees that there is a fairly good agreement, thus supporting the hypothesis of a different breakability of chromosomes in various stages of spermiogenesis.

V. DISCUSSION.

The purpose of the present investigation was to study the cause or causes of the variation in the rates of dominant lethals previously presented by BONNIER and LÜNING (1950) and LÜNING (1952 a). The earlier investigation of the subject by BONNIER and LÜNING (1950) led to a tentative hypothesis supposing a different rate of restitution of the broken chromosomes as the cause of the different rates of dominant lethals. The present results on studies of hyperploid males were contrary to those expected from this hypothesis. Instead it was supposed that the variation was due to a different rate of chromosome breaks induced in different stages of spermiogenesis and also affected by the age of the males at treatment. The accuracy of this hypothesis was tested on the one hand by the occurrence of two different regression lines for hatchability when treating 0—1 and 6—7 days old males with various doses and, on the other hand, by elimination of X- and Y-

chromosomes in different periods after treatment of 0—1 day old males. The results are in agreement with the expectation. Hence, the hypothesis of a differential breakability of chromosomes in various stages of spermiogenesis and in males of different ages is well-founded.

Besides hyperploid males I also got other types of aberrations, *viz.* gene mutations in the three loci, *y*, *w* and *sn*, and gynandromorphs due to elimination of the X-ray treated X-chromosome.

On the basis of the present material no definite variation in the rates of gene mutations induced in males of different ages and in various stages of spermiogenesis has been established. There may possibly be some minor differences but to establish them one needs a much greater material. One may, however, conclude that there are no variations comparable to those reported above for breaks leading to dominant lethals and hyperploid males.

BONNIER, LÜNING and PERJE (1949) reviewed the origin of X-ray induced gynandromorphs, supposing that they were due to breaks in the X-chromosome which restituted in one of the chromatids but remained open in the other, with subsequent elimination. BONNIER and LÜNING (1951) demonstrated that the rates of gynandromorphs varied with the age of males at treatment and with the time from treatment to insemination. Treatment of newly hatched males that were mated immediately after treatment, gave the highest rate of gynandromorphs. This has not been confirmed in the present experiments where there was no significant variation between the rates of gynandromorphs in Ser. 64, 65, 75 and 84. Thus, one may conclude that the rates of breaks leading to gynandromorphs are the same in all stages of spermiogenesis and in males of different ages. Hence, there is a discrepancy between the conclusions drawn from the occurrence of hyperploid males and gynandromorphs. The result of BONNIER and LÜNING (1951) gives a still greater discrepancy.

BONNIER, LÜNING and PERJE (1949) and LÜNING (1951) suggested that gene mutations and gynandromorphs may be due to the same cause. As neither of these aberrations varies with the stages treated the suggestion is not contradicted by the present investigation.

It was stated above, first, that there is a discrepancy between the rates of breaks leading to dominant lethals and hyperploid males and those leading to gynandromorphs, and, secondly, that the suggested common origin of gynandromorphs and gene mutations is not contradicted. This indicates that there is — at least in some stages — induced breaks that are not associated with the occurrence of gynandromorphs

and gene mutations. It does not, however, exclude the possibility that breaks may precede gene mutations. As it is now stated that there are induced breaks that cannot induce gene mutations (visibles) it is of great interest to study their possible association with recessive lethals. In some experiments now proceeding this will be studied.

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SUMMARY.

(1) The present paper deals with the effect of X-rays on males of *Drosophila melanogaster*. In most of the experiments *y w sn* females were mated to wild type males (Oregon). In two series *y v f*; bw^{VA} /L^2 *l* females were mated to males with closed X-chromosome, X^{c^2}.

(2) The males were irradiated at a definite age, mostly 0—1 or 6—7 days old, with 960 r or a multiple of this dose.

(3) The males were mated immediately after irradiation and were then transferred to new virgin females at certain intervals. The mating periods were 1—6, 7—10 and 11—14 days after treatment. These periods are determined by the results from studies on dominant lethals.

(4) The mechanisms at work in producing dominant lethals are reviewed. It is suggested that hyperploid males arise by the same mechanism as some dominant lethals.

(5) It is shown that in 0—1 day old males 960 r will induce as many dominant lethals and hyperploid males in spermatozoa inseminated 7—10 days after treatment as 3840 r will in spermatozoa used the first 6 days. It is concluded that the rates of chromosome breaks is about the same in the two series and, consequently, that the rates induced chromosome breaks are not the same in all stages of spermiogenesis.

(6) The sensitivity is not only due to the stages of spermiogenesis treated but also to the age of males at treatment. 6—7 days old males have a much lower variation than 0—1 day old males between various stages of spermiogenesis.

(7) The rates of gene mutations (visibles) and gynandromorphs do not show a variation comparable to that of dominant lethals and hyperploid males.

(8) It is shown that from the 11th day after treatment spermatozoa are used that were in a meiotic or a premeiotic stage at treatment.

(9) The hypothesis of a different breakability of chromosomes in males of various ages and in various stages of spermiogenesis was tested by the occurrence of two distinct regression lines for hatchability when various doses were given to 0—1 and 6—7 days old males.

(10) The change in sensitivity due to the age of males at treatment is established in 2—3 days old males in spermatozoa used the first 6 days after treatment, but in spermatozoa inseminated 7—10 days after treatment the 2—3 days old males are intermediary to 0—1 and 6—7 days old males.

(11) It is shown that the rates of viable losses of X- and Y-chromosomes in spermatozoa inseminated 1—6 and 7—10 days after treatment of X^{c^2} males are in agreement with the hypothesis of a different breakability.

(12) The consequence of the discrepancy between the results of dominant lethals and hyperploid males *versus* gene mutations and gynandromorphs is discussed and it is supposed that — at least in some stages — breaks are induced that are not associated with gene mutations.

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INTERACTION OF CENTROMERE AND CYTOPLASM IN CHROMOSOME ELIMINATION IN DROSOPHILA MELANOGASTER

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I. INTRODUCTION.

THE production by x-rays of gynandromorphs in *Drosophila melanogaster* was studied in two recent papers (BONNIER, LÜNING and PERJE, 1949; BONNIER and LÜNING, 1951). In this connection there was also made a comparison between the rates of gynandromorphs due to the elimination of the maternal or of the paternal X-chromosome; and the investigations were extended to unirradiated material as well. We made use of two different stocks, viz. *w sn* and *y f car*, and it was found that the spontaneous rates of maternal and paternal eliminations were about equal in the progeny of *w sn* females×*y f car* males, but that there occurred many more gynandromorphs due to paternal than due to maternal eliminations in the progeny of the reciprocal cross (BONNIER and LÜNING, 1951, Table 1). As the 2X individuals in these progenies were genotypically similar, it was suggested »that in different strains, differences in the egg cytoplasm act differentially on the chance that chromosomes from entering spermatozoa will be eliminated during ontogeny». The purport of the present article is to elucidate this problem further. The experiments reported below include thus the spontaneous production of gynandromorphs only.

II. THE EXPERIMENTS.

Table 1 summarizes all our experiments on the spontaneous production of gynandromorphs. For simplification the *w sn* strain is called A and the *y f car* strain is called B. The cross No. 1 is the same as that reported in Table 1 of our previous paper (BONNIER and LÜNING, 1951). As may be seen in that paper, a gradation was made of the certainty with which an aberrant fly was to be regarded as a gynandromorph or not. We classified them in three types, type 1 including certain gynandromorphs, type 2 probable gynandromorphs, and type 3 possible

gynandromorphs (for the definition of these three types, see p. 470 of the cited paper). In the table the total number of detected gynandromorphs of types 1 and 2 is found in columns 7 and 8. In case there also occurred gynandromorphs of type 3 the totals of types 1, 2 and 3 are given in brackets. Below the actual numbers in these columns the relative frequencies per 10^4 are given. In the significance columns, 9, 10 and 11, the figures within brackets refer as well to the totals of types 1, 2 and 3, whereas the figures without brackets refer to the totals of types 1 and 2 only.

Of the progeny of cross No. 2, 181411 were those given in Table 1 of the previous paper (BONNIER and LÜNING, 1951) whereas the remaining 38464 were raised simultaneously with those of cross No. 3. But as the results within these 38464 were just similar to the previous ones they are included in cross No. 2.

Cross No. 3 was started in order to test the assumed influence of the cytoplasm. Our intention was thus to produce a strain with the same genome as our original *y f car* strain (B genome) but with the cytoplasm of the *w sn* strain (A cytoplasm). To do this we used a stock containing the so-called Muller 5 and Muller 1 chromosomes which practically inhibit crossing over in the chromosomes 1, 2 and 3 and which also contain dominant marker genes in all these chromosomes. If the complex of these chromosomes is symbolized by the letter I, the progeny of A females and I males is A/I. Such females were crossed to B males and daughters showing the I markers — thus B/I animals with A cytoplasm — were again crossed to B males. In the next generation B females without I markers thus could be supposed to contain the unchanged chromosomes 1, 2 and 3 from the B strain but with the cytoplasm of the A strain. As no markers were used for chromosome 4, it is not certain that the whole genome of the B strain actually was introduced in A cytoplasm. But, with regard to the low rate of crossing over in the small chromosome 4, it must be approximately true to label, as is done in the table, the females of cross No. 3 as possessing the B genome and the A cytoplasm. All experiments shown in the table had to be repeated several times before the necessary order of magnitude was reached; and, in the case of cross No. 3, after the production of the first females with B genome and A cytoplasm their number had to be increased during a few generations. However, if the cytoplasm contains autonomous constituents which are transmitted from mother to offspring, all females used in this cross should contain the B genome and such constituents from the A cytoplasm. But, as may be seen from the

TABLE 1. *Rates of spontaneous gynandromorphs.*

A=*w sn* stock; B=*y f car* stock. In columns 7—11 the figures without brackets refer to gynandromorphs of types 1 and 2; and with brackets to gynandromorphs of types 1, 2 and 3 (for description of these types, see text and BONNIER and LÜNING, 1951, p. 470). Columns 7 and 8 include, for each separate cross, two lines: the upper line gives actual numbers and the lower line relative frequencies per 10^4. For computation of the significance levels in columns 9, 10, and 11 the method of angular transformation is used. If an observed proportion is denoted by p, this method consists — when p is such a small fraction as in the present case — of using $\sqrt{p}$ as a normal deviate with variance=$^1/_{4n}$, where n is the total from which p is computed.

Cross No.	Genome		Cytoplasm	Centromere of patern. X-chrom.	Total 2X progeny	Gynandromorphs		Significance, P, of difference between rates of patern. and matern. eliminations	Significance, P, of differences between rates of gynandromorphs when compared with rates in cross No. 1	
	matern.	patern.				maternal	paternal		maternal	paternal
1	2	3	4	5	6	7	8	9	10	11
1	A	B	A	B	221405	33 (34) 1,49 (1,54)	23 (31) 1,04 (1,40)	0,18 (0,70)		
2	B	A	B	A	219875	23 (24) 1,05 (1,09)	55 2,50	0,0002 (0,0004)	0,19 (0,19)	0,0002 (0,008)
3	B	A	A	A	41847	2 0,48	14 3,35	0,001	0,05 (0,04)	0,0024 (0,016)
4	A	B(X)	A	A	121149	10 0,83	9 0,74	0,80	0,08 (0,06)	0,38 (0,07)
5	B	A(X)	B	B	112492	12 1,07	6 0,53	0,15	0,31 (0,26)	0,11 (0,013)

table, the same surplus of paternal eliminations occurred as in the cross No. 2. Hence, this surplus could not be caused by such constituents alone.

We therefore tried to study the influence of the centromeres. F_1 females from cross between A and B strains, i. e. *w sn/y f car* females, were outcrossed, and among the progeny *y f* and *w sn car* sons were isolated. (To find *w sn car* males, a fairly large number of sons having white eyes and singed bristles were individually progeny tested and from this *w sn car* males could be secured.) Before making use of these males — which in each of the two crosses 4 and 5 originated from one single male — their number was increased by crossing them to double-attached-X females, and this procedure was repeated for the necessary number of generations. The autosomes of the males which finally were used in the experiments, were thus made up of autosomes from the double-attached stock as well as from A and B, respectively. The *y f* males contained most of the X-chromosome of the B strain [in the table labelled as B(X) genome] and the A centromere; and the *w sn car* males contained most of the X-chromosome of the A strain [A(X) genome] and the B centromere. They were respectively crossed to females of the A strain (cross No. 4) and to the B strain (cross No. 5). As the figures in column 9 of the table show, the proportion between paternal and maternal eliminations did not significantly deviate from 1.

Before going further the following point should be stressed. A surplus of gynandromorphs due to paternal eliminations could be caused by two different events. If there did exist »normal» rates of eliminations, a comparison between the rates in a special cross and these »normal» rates could either show an increase of paternal eliminations or a decrease of maternal ones. The result would in both cases be a surplus of paternal eliminations in this special cross. Unfortunately, however, we have no such »normal» standard to make comparison with. But as cross No. 1 was our original cross and as it gave a fairly good agreement to the 1 : 1 proportion between paternals and maternals, we have used this cross for comparisons. Columns 10 and 11 in the table show the significances of the differences between the rates in crosses No. 2 to No. 5 and those in this standard cross No. 1.

Looking now to the significance columns 9, 10 and 11, we find that crosses No. 2 and No. 3 — which are the only ones which give significant differences according to column 9 — also according to column 11 show significantly more paternal eliminations than does the standard cross No. 1. From this it follows that it is not the significantly lower

rate of maternal eliminations in cross No. 3 (column 10) that is the cause of the surplus of paternal eliminations in this cross. (The cause of the low rate of maternal eliminations in cross No. 3 is unknown.) Apart from these significant differences it is only the lower rate of paternal eliminations in cross No. 5 (P=0,013, column 11) whose significance is worth mentioning. But this refers to the comparison with cross No. 1 when all three types of gynandromorphs are included. It is not unlikely, however, that some of the type 3 gynandromorphs were not real gynandromorphs, and this comparison is therefore of less interest.

III. CONCLUSIONS.

By comparing the frequencies in the different crosses, the following suggestions seem to be valid.

In crosses Nos. 1 and 2 the flies have the same type of genome; hence, the surplus of paternal eliminations in cross No. 2 cannot be caused by the genome only.

In crosses Nos. 2 and 3 the flies have similar genomes but dissimilar cytoplasms. As in both these crosses similar excesses of paternal eliminations are found, the cytoplasm alone cannot differentiate cases with high from those with »normal» rates of such eliminations.

The comparison of crosses Nos. 2, 3 and 4 indicates that paternal chromosomes with A centromeres more often are eliminated if they are introduced in a cytoplasm — whether this is A or B cytoplasm — which is under the control of B genome (the maternal part) than when the cytoplasm is under control of A genome.

Crosses Nos. 1 and 5, which give no statistically significant differences in the rates of maternal and paternal eliminations, indicate that paternal X-chromosomes with B centromeres are eliminated at a »normal» rate whether they are introduced in A or in B cytoplasm and whether these cytoplasms are under the control of A or of B genomes.

If the above conclusions are correct they show that different centromeres may be isolated in *Drosophila* which react differentially in certain genetical events.

SUMMARY.

(1) A study is made of the causes at work in the production, through chromosome elimination, of gynandromorphs in *Drosophila melanogaster*.

(2) Two different strains were used with different marker genes in their X-chromosomes, viz. *w sn* (here named the A strain) and *y f car* (here named the B strain). A series of experiments were made the results of which are summarized in Table 1. The different rates of maternal and of paternal eliminations have led to assume the validity of the suggestions made in the preceding section.

(3) These suggestions include the assumption that different paternal centromeres may act differentially according to the maternal cytoplasm into which they are introduced and according to the maternal genome under the control of which the cytoplasm is produced.

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HALMAUFBAU UND STANDFESTIGKEIT BEI ERECTOIDES MUTANTEN DER GERSTE

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(With a Summary in English)

EINLEITUNG.

DIE sogenannten *Erectoiden* gehören zu den auffälligsten morphologischen Mutanten der Gerste vom *nutans*-Typus. Neben vereinzeltem spontanen Auftreten wurden diese Mutanten in verschiedenen Sorten und durch verschiedene Autoren unabhängig zahlreich durch Bestrahlung induziert (GUSTAFSSON, 1947; FREISLEBEN und LEIN, 1944; STUBBE und BANDLOW, 1947). Die von GUSTAFSSON und Mitarbeitern isolierten 50 *erectoides*-Typen sind alle durch aufrechte, kurze und kompakte Ähre sowie grosse Standfestigkeit charakterisiert. Zumeist ist diese gegenüber der Muttersorte grössere Standfestigkeit mit kürzerem Halm verbunden, jedoch haben einige gleiche Strohlänge, ja im Falle *erectoides* 14 ist bei extrem kurzer Ähre das Stroh eindeutig länger (GUSTAFSSON, 1947, p. 22). Das weist darauf hin, dass auch andere morphologische und anatomische Eigenschaften die Standfestigkeit beeinflussen.

Die Vitalität der *Erectoiden*, gemessen am Kornertrag, ist unter »Standardbedingungen» teilweise geringer, z.T. gleich oder auch grösser wie die der Ausgangssorte (FREISLEBEN und LEIN, 1944; GUSTAFSSON, 1947). Unter veränderten Aussenbedingungen aber können auch die Mutanten mit Ertragssenkung den Ertrag der Mutterlinie erreichen oder überschreiten (GUSTAFSSON und NYBOM, 1950; GUSTAFSSON, 1952). Die hier untersuchte Mutante *erectoides* 23 aus der Svalöfer Hochzuchtsorte *Bonus* hat die oben beschriebenen Eigenschaften, liegt im Ertrag bei Düngung mit 250 Kg Salpeter/ha ca. 5 % niedriger, bei 500 kg Salpeter/ha ungefähr so hoch wie *Bonus*. Dem und dem Ergebnis der unten dargelegten Strohstärkebonitierungen zufolge kann *erectoides* 23 höchste Stickstoffdüngung besser ausnützen (GUSTAFSSON, unpubl.).

Vorliegende Untersuchung soll klarlegen, welche morphologischen und anatomischen Eigenschaften verändert sind bei Pflanzen, die sich

vom Vergleichsmaterial durch ein Gen unterscheiden, das *erectum*-Habitus und damit grosse Standfestigkeit bedingt, sowie durch eine gegenüber oekologischen Faktoren veränderte Reaktionsnorm.

Die Standfestigkeit wird durch äussere und innere Ursachen festgelegt. Zu den inneren, d. h. vom Genotypus bestimmten, Ursachen dürfen wir die folgenden Eigenschaften rechnen (z. T. nach KRAUS, 1912):

1. Der morphologische Aufbau des Halmes: Pflanzenlänge, Länge und Zahl der Internodien, Grösse des Halmquerschnittes, Halmwanddicke.

2. Der anatomische Bau der Halmwand: Anordnung der Festigungselemente, Zellwanddicke des Sklerenchyms, Anordnung der mizellaren Struktur, Zellgrösse.

3. Die Art, Menge und Einlagerung der zur Festigung der Zellmembranen dienenden Stoffe: z. B. Lignin.

4. Der Entwicklungsverlauf der Halme: z. B. Zeitpunkt der Verholzung des Gewebes.

5. Die Ausbildung des Wurzelsystems.

6. Die morphologische Beschaffenheit der Ähre.

Die Standfestigkeit wird durch die Ausbildung jeder dieser einzelnen Eigenschaften bedingt, zugleich aber durch das Zusammenspiel aller dieser in einem bestimmten, unter physikalischen Gesetzen stehenden, mechanischem System.

MATERIAL UND METHODIK.

Die Untersuchung wurde an Pflanzen aus Parzellen der vergleichenden Ertragsversuche der Mutationsabteilung des Sveriges Utsädesförening, Svalöf ausgeführt. Die Parzellen hatten die höchste Stickstoffdüngung (500 kg Salpeter/ha), da Standfestigkeitsbonitierungen des Jahres 1951 bei unterschiedlicher Düngung eindeutig hier die grössten Unterschiede zwischen Muttersorte und Mutante zeigen. Das Material wurde zufallsgemäss aus der Mitte des Feldes entnommen, und zwar je Pflanze ein Haupthalm. Besonders wurde darauf geachtet nur Individuen mit für Muttersorte bzw. Mutante charakteristische Ähren zu verwenden. Die Bestimmung der Standfestigkeit, wie gleichzeitige morphologische und anatomische Bearbeitung erfolgte kurz vor der Ernte und zum Vergleich wurde einiges Material 4 Wochen vorher fixiert.

Methoden zur direkten Bestimmung der Standfestigkeit gründen sich auf Korrelation von Bruchfestigkeit, Gewicht von Halmstücken und Strohstärke. Ein den natürlichen Bedingungen besser angepasste

Grösse ist die Biegungsfestigkeit, zu deren Messung Halmdynamometer konstruiert worden sind (ROEMER und RUDORF, 1950 u. dort zit. Literatur). Als Mass für Biegungsfestigkeit kann auch die Senkungsgrösse eines horizontal eingespannten Halmstückes verwendet werden. Die Senkung S des freien Endes ist (nach SCHWENDENER, 1874) gegeben durch die Formel: $S = \frac{l^3 P}{3 WE}$ (l=Länge, P=angehängtes Gewicht, W=Mass

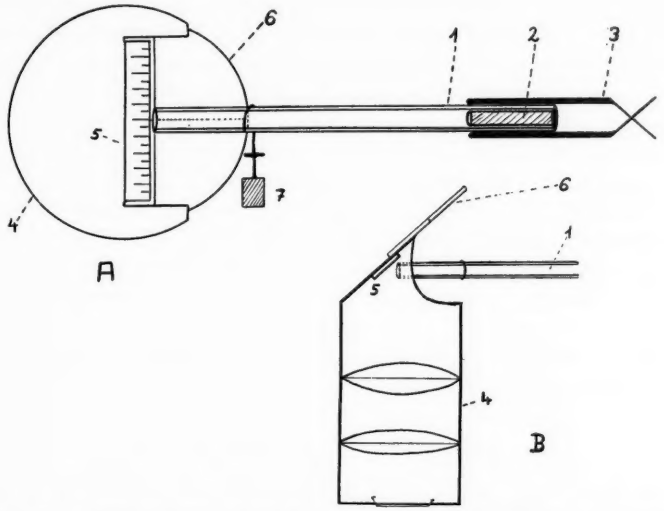


Fig. 1. Schema der Anordnung zur Bestimmung der Senkungsgrösse. A: von der Seite, B: von oben. — 1, das zu untersuchende Internodium, — 2, in den Hohlraum passendes Glasrohr (1 cm), — 3, Klammer, — 4, Okular mit 8facher Vergr., — 5, Skala, — 6, Scheibchen mit Fadenkreuz, — 7, Gewicht.

des Biegungsmomentes, E=Elastizitätsmodul). Die Senkungsgrösse wurde folgendermassen bestimmt (vergl. Fig. 1): Das zu bestimmende Internodium wird am einen Ende mit einem genau in den Hohlraum passendem Glasrohr verstärkt und 1 cm in eine am Stativ veränderlich befestigte Klammer eingespannt. In entsprechender Höhe wird ein Spezialokular mit eingebauter Skala an einem zweiten Stativ angebracht. Ein Scheibchen mit Fadenkreuz ermöglicht parallaxenfreie Ablesung. Das freie Ende wird in 1 cm Entfernung mit Gewichten belastet. Durch das Okular lässt sich auf der Skala der Unterschied vor und nach der Belastung auf 0,05 mm genau ablesen. Die Bestimmung der Senkungsgrösse darf nur innerhalb der Elastizitätsgrenze erfolgen und nach jeder

Belastung ist zu prüfen, ob der Halm wieder in die Ausgangslage zurückkehrt. Differenzen der Werte, die bei Wechsel des freien Endes oder Drehung der Halmstücke entstehen, sind zumeist gering und dürfen hier vernachlässigt werden. Die Bestimmung erfolgte für alle Internodien einer Pflanze, in ganzer Länge von Knoten zu Knoten, getrennt. Die angehängten Gewichte waren 1, 2, 5, 10, u. 20 g, jeweils bis zur Überschreitung der Elastizitätsgrenze. Die Proportionalität zwischen Gewicht und Senkung war gut. Erwies sich ein Internodium zu lang, um bei 1 g Belastung einen Ausschlag innerhalb des Elastizitätsbereiches zu geben, so wurde in zwei Hälften gemessen und das Mittel gezogen. Die gefundenen Werte wurden nachträglich auf die einheitliche Länge von 10 cm reduziert.

Zur anatomischen Untersuchung gelangten Paraffinschnitte nach Fixierung in Navashin's Gemisch und Färbung mit Safranin—Lichtgrün. Infolge der Zusammensetzung der Halmwand aus verholztem und unverholztem Gewebe, und infolge des Hohlraumes zerissen die Schnitte sehr leicht. Befriedigende Resultate brachte nur ein Eintauchen der angeschnittenen Paraffinblöcke in destilliertes Wasser für mindestens 7 Tage.

Die statistische Bearbeitung ist nach den Methoden der Varianzanalyse durchgeführt (BONNIER und TEDIN, 1940).

DIE MORPHOLOGISCHE ANALYSE.

Als Grundlage zur vergleichenden Beurteilung der Standfestigkeit dienen Bonitierungen im Feldbestand unter Verwendung einer Skala von 1 (niederliegend) bis 10 (völlig aufrecht). *Bonus*-Gerste hat für den Zeitraum von 5 Jahren (1947—51) eine mittlere Standfestigkeit von 7,8, während für *erectoides* 23 im gleichen Zeitraum 9,3 festzustellen ist. Noch anschaulicher wird die Differenz, wenn man die Zahlen des Jahres 1951 unter Berücksichtigung der unterschiedlichen Düngung betrachtet:

Salpeter kg/ha		200	300	400	500	M
Standfestigkeit	*Bonus*	8,5	6,5	5,5	*3,0*	5,9
	erectoides	9,0	8,5	7,5	*6,5*	7,9

Bei jeder Düngung ist *erectoides* standfester, jedoch bei maximaler Stickstoffgabe wird der Unterschied am grössten. *Erectoides* ist nicht so empfindlich gegen hohe Stickstoffdüngung wie *Bonus*-Gerste.

Für *Bonus* ist an den untersuchten Pflanzen eine mittlere Strohlänge von $82{,}9 \pm 0{,}6$ cm (n=6), für *erectoides* 23 von $75{,}5 \pm 2{,}0$ cm (n=6) ermittelt. Wenn auch die Zahl der Messungen klein, so ergibt sich doch für die Differenz eine statistische Sicherung ($v^2 = 12{,}85$; $0{,}01 \geqslant P > 0{,}001$). Welcher Teil des Halmes von der Verkürzung betroffen wird, kann uns der Vergleich der Internodienlängen, gemessen von Knoten zu Knoten, zeigen. Da auch innerhalb einzelner Gerstensorten eine Variabilität in der Anzahl der Internodien besteht, ist es notwendig einen Fixpunkt zu bestimmen, von dem ausgehend die einzelnen Internodien verglichen werden. KÖNIG (1928) hat gezeigt, dass das oberste Internodium eines 6 gliedrigen Halmes auch dem obersten eines 7 gliedrigen entspricht und in gleicher Weise das unterste des 6 gliedrigen dem zweiten des 7 gliedrigen. Diese Befunde konnten im Laufe unserer Messungen bestätigt werden und so ist hier die Ähre als Bezugspunkt gewählt. Aus praktischen Gründen werden die Internodien vom obersten aus mit 1 bis 5 bezeichnet. (Von den 6 und 7 gliedrigen Halmen sind ein bzw. zwei unterste Internodien bei der Analyse ausgeschaltet.)

Länge in cm.

Internodium	1	2	3	4	5
Bonus	$22{,}06 \pm 0{,}60$ n = 10	$17{,}90 \pm 0{,}78$ n = 10	$12{,}48 \pm 0{,}78$ n = 5	$12{,}54 \pm 0{,}34$ n = 10	$9{,}04 \pm 1{,}06$ n = 5
erectoides 23	$24{,}22 \pm 1{,}01$ n = 10	$18{,}45 \pm 0{,}36$ n = 10	$11{,}80 \pm 0{,}80$ n = 5	$10{,}52 \pm 0{,}23$ n = 10	$5{,}30 \pm 0{,}64$ n = 5

Internodium 1+2: Variationsursache: *Bonus/erectoides* $v^2 = 1{,}423$; $0{,}2 > P \geqslant 0{,}05$
Internodium 3+4+5: » : » $v^2 = 20{,}63$; $0{,}01 \geqslant P > 0{,}001$

Die oberen Internodien dürften gleich lang sein. Die geringere Länge der basalen Internodien bedingt den kürzeren *erectoides*-Halm. Dies kann als Ursache für grössere Standfestigkeit gewertet werden; ist es die alleinige Ursache, so müssen alle übrigen morphologischen Eigenschaften in gleicher Weise ausgebildet sein.

Zunächst wurde für alle oben gemessenen Internodien die Senkungsgrösse in mm/10 cm Halm bestimmt, bei einem angehängten Gewicht von 1 g (siehe Tabelle 1).

Innerhalb beider Sorten nehmen die Senkungsgrösse von der Basis zur Spitze des Halmes zu, d.h. die Biegungsfestigkeit nimmt ab, bei *erectoides* aber wesentlich weniger. Die obersten Internodien sind im Vergleich mit der Muttersorte statistisch (mit P beinahe = 0,001) gesichert

TABELLE 1. *S in mm.*

Internodium	1	2	3	4	5
Bonus	$1{,}77 \pm 0{,}15$ n = 10	$0{,}47 \pm 0{,}09$ n = 10	$0{,}30 \pm 0{,}03$ n = 5	$0{,}29 \pm 0{,}04$ n = 10	$0{,}26 \pm 0{,}04$ n = 5
erectoides 23	$1{,}14 \pm 0{,}09$ n = 10	$0{,}26 \pm 0{,}04$ n = 10	$0{,}26 \pm 0{,}02$ n = 5	$0{,}31 \pm 0{,}10$ n = 10	$0{,}18 \pm 0{,}12$ n = 5

Internodium 1+2: Varianzursache: *Bonus/erectoides*, $v^2 = 12{,}379$, $0{,}01 \gg P > 0{,}001$
Internodium 3+4+5: » : » , $v^2 = 1{,}250$, $P \gg 0{,}2$

TABELLE 2.

Gesamte Querschnittsfläche des Halmes; F in mm².

Internodium	1	2	3	4	5
Bonus	$3{,}623 \pm 0{,}169$ n = 10	$8{,}233 \pm 0{,}366$ n = 10	$8{,}171 \pm 0{,}666$ n = 6	$8{,}454 \pm 0{,}448$ n = 10	$6{,}226 \pm 0{,}502$ n = 5
erectoides	$4{,}119 \pm 0{,}178$ n = 10	$9{,}099 \pm 0{,}333$ n = 10	$8{,}847 \pm 0{,}614$ n = 6	$8{,}416 \pm 0{,}500$ n = 10	$5{,}583 \pm 0{,}660$ n = 5

Internodium 1+2: Varianzursache: *Bonus/erectoides*, $v^2 = 4{,}268$, $P \approx 0{,}05$
Internodium 3+4+5: » : » , $v^2 = 0{,}025$, $P \gg 0{,}2$

Querschnittsfläche des Halmhohlraumes: f in mm².

Internodium	1	2	3	4	5
Bonus	$2{,}250 \pm 0{,}115$ n = 10	$5{,}635 \pm 0{,}243$ n = 10	$5{,}495 \pm 0{,}450$ n = 6	$5{,}277 \pm 0{,}258$ n = 10	$3{,}047 \pm 0{,}366$ n = 5
erectoides	$2{,}608 \pm 0{,}195$ n = 10	$6{,}232 \pm 0{,}264$ n = 10	$5{,}868 \pm 0{,}427$ n = 6	$4{,}747 \pm 0{,}445$ n = 10	$2{,}029 \pm 0{,}471$ n = 5

Internodium 1+2: Varianzursache: *Bonus/erectoides*, $v^2 = 3{,}17$, $0{,}2 \gg P > 0{,}05$
Internodium 3+4+5: » : » , $v^2 = 0{,}11$, $P \gg 0{,}2$

Fläche der Halmwand: F—f in mm².

Internodium	1	2	3	4	5
Bonus	$1{,}373 \pm 0{,}064$ n = 10	$2{,}598 \pm 0{,}243$ n = 10	$2{,}675 \pm 0{,}751$ n = 6	$3{,}177 \pm 0{,}218$ n = 10	$3{,}180 \pm 0{,}274$ n = 5
erectoides	$1{,}511 \pm 0{,}062$ n = 10	$2{,}867 \pm 0{,}173$ n = 10	$2{,}979 \pm 0{,}719$ n = 6	$3{,}668 \pm 0{,}217$ n = 10	$3{,}554 \pm 0{,}228$ n = 5

Internodium 1+2: Varianzursache: *Bonus/erectoides*, $v^2 = 2{,}73$, $0{,}2 > P > 0{,}05$
Internodium 3+4+5: » : » , $v^2 = 0{,}81$, $P \gg 0{,}2$

biegungsfester. Wir dürfen also Unterschiede der Halmstruktur in diesen beiden Internodien erwarten, nicht aber in den basalen. Den grössten Einfluss auf die Biegungsfestigkeit hat die Querschnittsfläche des Halmes und die Dicke der Halmwand. Handschnitte aus der Mitte der Internodien wurden mit dem Zeichenspiegel in 45 facher Vergrösserung gezeichnet und nachträglich die Gesamtfläche und der Hohlraum ausplanimetriert. Die erhaltenen Flächen sind auf natürliche Grösse reduziert. Überblicken wir die Werte von Tabelle 1, so lässt sich ableiten: Die Querschnittsflächen des Halms sind für beide Formen in den mittleren Internodien am grössten und nehmen gegen die Basis wie gegen die Spitze hin ab. Das Verhalten des Hohlraumes entspricht ganz dem der Gesamtquerschnitte, ebenfalls Abnahme von der Mitte des Halmes gegen Basis und Ähre. Hingegen die Fläche und damit die Dicke der Halmwand ändert sich unabhängig von den zuletzt besprochenen Grössen, sie nimmt übereinstimmend in *erectoides* und *Bonus* von unten nach oben ab. Im Hinblick auf die Festigkeit ist also das allgemeine mechanische System des Aufbaues beider Halmtypen gleich.

In den einzelnen Internodien aber besteht ein Unterschied zwischen *Bonus* und *erectoides* im Grade der Ausbildung jener Eigenschaften, die dieses System bedingen. Das 3., 4. und 5. Internodium ist von gleichem morphologischen Aussehen; es lässt sich keine Differenz der beiden Sorten nachweisen (Alle P Werte liegen weit über 0,2). Im 1. und 2. Internodium bemerkt man eine grössere Querschnittsfläche für *erectoides,* was bei gleicher Wandstärke eine stärkere mechanische Beanspruchung zulässt. Die Parallelität zwischen den Messungen der Senkungsgrösse und denen der Querschnittsflächen ist auffallend. *Erectoides* und *Bonus* haben übereinstimmend gleiche Biegungsfestigkeit, gleiche Gesamt- und Hohlraumquerschnittsfläche in den 3 basalen Internodien, während die oberen Internodien von *erectoides* grössere Biegungsfestigkeit und grössere Gesamt-, wie Hohlraumquerschnittsfläche aufweisen. Die statistische Bearbeitung erlaubt keine Sicherung der Unterschiede, da aber bei allen untersuchten Individuen sich diese Tendenz bemerkbar macht und bei Betrachtung im Feldbestand gerade die grössere Dicke der oberen Halmglieder immer wieder beobachtet werden konnte, so möchte ich doch die Differenz als reell betrachten. Die Senkungsgrösse lässt sich auch annähernd aus den bestimmten Flächen berechnen nach der Formel $S = \frac{l^3 P}{3\,WE'}$, wobei W, das Mass des Biegungsmomentes, gegeben ist durch:

$$W = \frac{F\, r_1^2 + r_2^2}{4}.$$

Unter Voraussetzung gleichen Elastizitätsmoduls (was nicht näher geprüft wurde) verhalten sich $\frac{S_{Bonus}}{S_{erect.}} = \frac{W_{erect.}}{W_{Bonus}}$. Die erhaltenen Werte $\frac{S}{S'}$ aus der experimentellen Bestimmung, sowie die aus der Fläche errechneten $\frac{W'}{W}$ stimmen in Internodium 1 und 2 grössenordnungsmässig

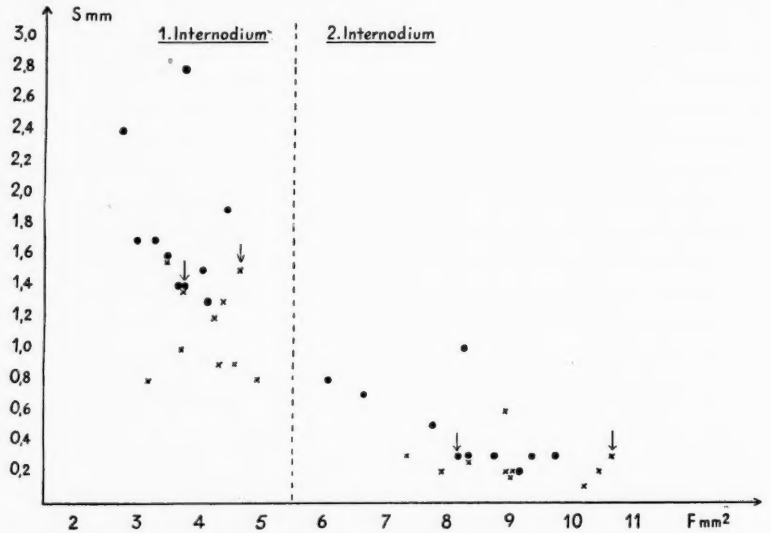


Fig. 2. Korrelation von Senkungsgrösse und Querschnittsfläche in den beiden obersten Internodien. — *Bonus*: .. *Erectoides* 23: ×.

überein, während in den unteren Internodien starke Abweichung auftritt. Hier ist nicht so sehr die Querschnittsfläche von Bedeutung, als vielmehr die Wandstärke.

Zur Veranschaulichung dieser Korrelation von S und F sei auf Fig. 2 verwiesen. Zur Berechnung eines Korrelationskoeffizienten sind bei der grossen Streuung die Anzahl der Werte zu klein. Die Werte von *erectoides* liegen im Wesentlichen unter denen von *Bonus*; es ist daher Grund zur Annahme, dass es sich um zwei verschiedene Funktionen handelt. Mit anderen Worten sind zugleich weitere Veränderungen als Grund für die grössere Biegungsfestigkeit zu suchen, was die anatomi-

sche Untersuchung bestätigt hat. Auch die starken Abweicher von der Regressionslinie dürften darauf zurückzuführen sein, dass in diesen Halmen anatomische Eigenheiten die Senkungsgrösse beeinflussen.

Eventuell ist eine Verdickung der Halmwand in den oberen Internodien von *erectoides* in Betracht zu ziehen. (Siehe Werte F—f.) Bei gleicher Querschnittsfläche ist ein Hohlzylinder innerhalb gewisser Grenzen um so biegungsfester je dünner die Wand, unter Voraussetzung völliger Homogenität derselben. Eine Verdickung der Halmwand würde also mit Rücksicht auf die Biegungsfestigkeit einer Flächenvergrösserung entgegenwirken. Die Halmwand ist in keiner Weise homogen. Da zudem aber gerade, wie wir weiter unten sehen werden, *erectoides* grössere Zellen und Zellumina besitzt, ist die Wandstruktur mechanisch gesehen gegenüber *Bonus* so verändert, dass der geringe Unterschied in der Wanddicke zumindest ausgeglichen wird.

Es scheint der Schluss nicht abwegig, das der vergrösserte Halm- und Hohlraumquerschnitt eine Teilursache für die grössere Biegungsfestigkeit der oberen Internodien von *erectoides* 23 ist.

DIE ANATOMISCHEN BEFUNDE.

Zur Bestimmung der anatomischen Unterschiede kurz vor der Ernte wurden zwei charakteristische Individuen der morphologisch Untersuchten zufallsgemäss ausgewählt. In Fig. 2 sind die für diese geltenden Punkte durch Pfeile markiert. Es ist danach die Fläche von *erectoides* im 1. und 2. Internodium grösser, im Ganzen liegt der *erectoides*-Halm dem von *Bonus* näher in den Eigenschaften der Senkungsgrösse und Querschnittsfläche als nach Fig. 2 dem Durchschnitt von *erectoides* entspricht. Die Schnittserien des 4 Wochen vor der Ernte fixierten Materials stimmen in der Anordnung der Strukturen und dem Grad der Verholzung mit dem bei voller Reife untersuchten überein, bis auf die Degeneration des Assimilationsparenchyms in letzterem. Es ist also berechtigt alles gemeinsam zu behandeln. Von den Schnittserien wurden Mikrophotographien hergestellt, auf diesen die verschiedenen Messungen ausgeführt und die Werte auf natürliche Grösse reduziert.

Fig. 3 möge noch einmal die grössere Querschnittsfläche von *erectoides* im 1. und 2. Internodium veranschaulichen. Um eine Auffassung über die Änderung der Querschnittsfläche und Strukturen innerhalb eines Internodiums zu gewinnen, sind Schnitte in 6 verschidenen Höhen des 1. Internodiums ausplanimetriert:

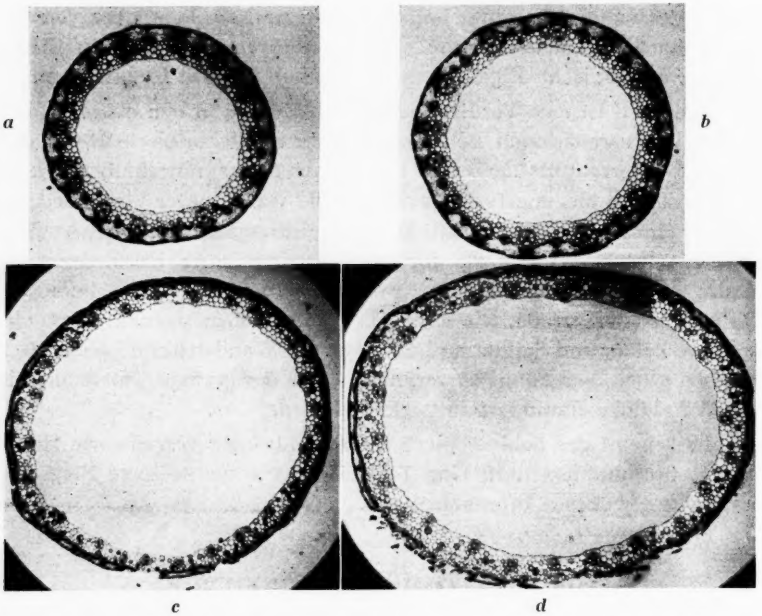


Fig. 3. Querschnitt im oberen Teil des 1. Internodiums, *a*: *Bonus*, *b*: *erectoides* 23. Querschnitt in der Mitte des 2. Internodiums, *c*: *Bonus*, *b*: *erectoides* 23. (Vergr. *a*, *b*: 22×; *c*, *d*: 15×.)

Fläche in 1000 μ².

Ähre ← → Basis des 1. Internodiums

Schnitthöhe	I	II	III	IV	V	VI
Bonus	1109	1229	1876	3026	3353	3681
erectoides 23	1737	2024	2394	3854	4336	4548

Die Gesamtquerschnittsfläche des Halmes nimmt innerhalb des 1. Internodiums in beiden Typen von der Basis gegen die Ähre gleichmässig ab. — Im 2. und 3. Internodium haben wir eine Abnahme von der Mitte gegen die Basis und Spitze, während schliesslich im 4. und 5. Internodium das ganze Glied gleich dick ist. Die gleiche Gesetzmässigkeit haben in verschiedenen Gerstensorten auch KRAUS (1912) und KÖNIG (1928) gefunden.

Die Grösse, sowie Anordnung der einzelnen Gewebe und Gefässbündel ändert sich in gleicher Weise bei *Bonus* und *erectoides*, zusammen mit der Vergrösserung bzw. Verkleinerung der Querschnitts-

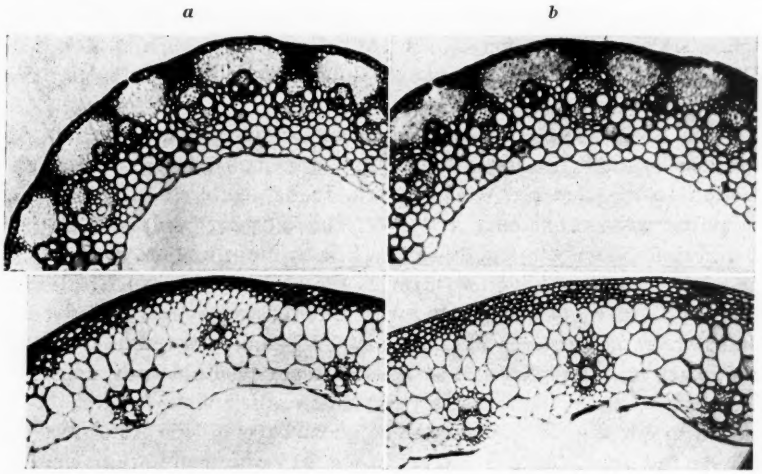


Fig. 4. Teil eines Schnittes im oberen Teil des 1. Internodiums. *a*: *Bonus*; *b*: *erectoides* 23.
Teil eines Schnittes in der Mitte des 2. Internodiums. *c*: *Bonus*; *d*: *erectoides* 23.
(Vergr. *a*, *b*, *c*, *d*: 73×.)

fläche. Es ist deshalb beim Vergleich nicht entscheidend in welcher Höhe innerhalb eines Internodiums der Schnitt geführt wird, sondern nur dass er aus verhältnismässig denselben Höhen genommen wird.

Folgende anatomische Eigenschaften sind untersucht: 1. Zahl der Gefässbündel, 2. Abstand der Gefässbündel vom Innenrand des Parenchyms, 3. Lage und Dicke des Sklerenchymringes, 4. Tangentialer und radialer Durchmesser der Gefässbündel, 5. Zellgrösse.

Die Zahl der Gefässbündel (es werden nur die grossen im Parenchym eingebetteten berücksichtigt) ist für *Bonus*, wie *erectoides* in den einzelnen Internodien gleich.

Internodium	1	2	3	4	5
Bonus	$14{,}4 \pm 0{,}8$ $n = 9$	$26{,}1 = 0{,}5$ $n = 10$	$24{,}5 \pm 0{,}6$ $n = 6$	$25{,}6 \pm 0{,}5$ $n = 10$	$22{,}2 \pm 1{,}1$ $n = 6$
erectoides	$14{,}1 \pm 0{,}5$ $n = 9$	$25{,}6 \pm 0{,}8$ $n = 10$	$25{,}0 \pm 0{,}8$ $n = 6$	$23{,}1 \pm 0{,}8$ $n = 10$	$22{,}2 \pm 1{,}4$ $n = 5$

Internodium 1+2: Varianzursache: *Bonus*/*erectoides*, $v^2 = 1{,}145$, $P > 0{,}2$
Internodium 3+4+5: » : » , $v^2 = 0{,}02$, $P \gg 0{,}2$

Auch die ersten und zweiten Internodien mit den grösseren Querschnittsflächen haben keine vermehrte Gefässbündelanzahl. Das wird ebenfalls aus Fig. 3 deutlich, wo die Gefässbündel von *erectoides* weiter auseinander liegen.

Unterschiede in der Lage der Gefässbündel, gemessen an den Abständen von der Innenepidermis des Parenchyms, lassen sich in den oberen Internodien nicht nachweisen. In den unteren Internodien ist möglicherweise bei *erectoides* eine Verschiebung der Gefässbündel gegen die Peripherie eingetreten. Mit Sicherheit ist dies nicht entschieden.

Die Lage des Sklerenchymringes (vergl. Fig. 3 und 4) ist dieselbe, wie im Ganzen die Anordnung von Sklerenchym, Gefässbündel und Parenchym bei unserer Mutante nicht abgeändert ist. Die Dicke des Sklerenchymringes einschliesslich der äusseren Epidermis wurde an den Schnitten fortlaufend in bestimmten Abständen festgestellt. Im ersten Internodium sind die Sklerenchymstränge, die die grossen Gefässbündel mit der Epidermis (siehe Fig. 4 *a*, *b*) verbinden, in radialer Richtung vermessen.

Dicke des Sklerenchymringes in μ.
(n = Zahl der Einzelmessungen)

Internodium	1	2	3	4	5
Bonus	$55{,}8 \pm 2{,}0$ $n = 11$	$34{,}7 \pm 1{,}8$ $n = 45$	$33{,}0 \pm 2{,}0$ $n = 19$	—	$69{,}8 \pm 2{,}4$ $n = 26$
erectoides 23	$68{,}1 \pm 2{,}4$ $n = 8$	$54{,}3 \pm 2{,}2$ $n = 28$	$46{,}9 \pm 2{,}5$ $n = 21$	—	$75{,}1 \pm 3{,}0$ $n = 24$
	$t = 3{,}967$ $P = 0{,}001$	$t = 6{,}759$ $P \ll 0{,}001$	$t = 4{,}373$ $P < 0{,}001$		$t = 1{,}359$ $P \approx 0{,}2$

Im ersten Internodium sind auch Werte von dem tangentialen Durchmesser des Sklerenchymbalkens zwischen dem Assimilationsparenchym erhalten und diese weisen ähnliche Unterschiede auf. Das Sklerenchym nimmt also in den drei obersten Internodien von *erectoides* eine grössere Fläche ein. Fig. 4 *c*, *d* gibt ein gutes Beispiel für die Verhältnisse. Der dickere Sklerenchymring kann prinzipiell auf zweierlei Wegen entstanden sein, entweder durch Vermehrung der Zahl der Sklerenchymzellen oder bei konstanter Zellzahl durch Zellvergrösserung. Letzteres trifft hier zu. In Fig. 4 kann man die grösseren Zellumina im Sklerenchym von *erectoides* erkennen. Direkte Messung der Zellgrösse hat diese Auffassung bestätigt (vergl. unten).

Die Untersuchung der Gefässbündel hat für *erectoides* in den oberen Internodien eine grössere Querschnittsfläche ergeben. Es wurde der tangentiale Radius an der breitesten Stelle der Bündel vermessen einschliesslich der das Bündel umgebenden Sklerenchymzellen, wie auch der radiale Durchmesser median von der äusseren Grenze des Phloems bis und einschliesslich der das Xylem umgebenden Sklerenchymzellen. Tangentialer und radialer Durchmesser sind stark korreliert; ist der eine Durchmesser gross, so ist es auch der andere. Im Ganzen ist bei allen Internodien eine Vergrösserung der beiden Durchmesser in *erectoides* festzustellen. In den oberen Internodien sind die Unterschiede gesichert.

Tangentialer Durchmesser der Gefässbündel in μ.
(n = Zahl der Gefässbündel)

Internodium	1	2	3	4	5
Bonus	$96{,}2 \pm 2{,}3$ $n = 13$	$71{,}4 \pm 3{,}0$ $n = 24$	$97{,}1 \pm 3.0$ $n = 26$	—	$93{,}2 \pm 2{,}8$ $n = 23$
erectoides 23	$115{,}1 \pm 3{,}1$ $n = 14$ $t = 4{,}974$ $P < 0{,}001$	$98{,}7 \pm 3{,}3$ $n = 26$ $t = 6{,}089$ $P \ll 0{,}001$	$111{,}6 \pm 3{,}5$ $n = 24$ $t = 3{,}222$ $0{,}01 > P \gg 0{,}001$	—	$101{,}3 \pm 3{,}5$ $n = 18$ $t = 1{,}840$ $0{,}2 > P > 0{,}1$

Auch für die dickeren Gefässbündelstränge in *erectoides* möchte ich vermuten, dass als Ursache eine Vergrösserung der einzelnen Zellen anzusehen ist. Beim Vergleich von Fig. 4 *a*, *c* mit 4 *b*, *d* sind ja die Tracheen grösser in der Mutante, und auch die Siebzellen und die das Bündel einschliessenden Sklerenchymzellen.

Da alle anatomischen Unterschiede der Halmwand auf einer Zellvergrösserung zu beruhen schienen, wurde schliesslich versucht auch die Zellgrösse zu bestimmen. Dies ist bei einem aus so verschiedenartigen Zellen zusammengesetzten Organ wie die Halmwand der Internodien mit grossen Schwierigkeiten verbunden. Die Zellen der Gefässstränge und des Sklerenchyms mussten von vorneherein infolge ihrer grossen Variabilität ausscheiden. Das Parenchym weist die grössten zusammenhängenden Zellflächen auf und man erhält unter Berücksichtigung aller Zellen vom Innenrand des Sklerenchyms bis einschliesslich der Innenepidermis, eine ziemlich einheitliche mittlere Zellgrösse. Von einzelnen Schnitten wurden Flächen bestehend aus mindestens 35 Zellen ausplanimetriert, unter Ausschluss der von den Gefässbündel be-

deckten Flächen. Bei den verglichenen Individuen wurde allgemein zumindest eine Tendenz der Zellvergrösserung in den oberen Internodien von *erectoides* festgestellt. Einige Messungen mögen die Verhältnisse beleuchten:

Zellfläche in μ^2.
(n = Zahl der Zellen)

Internodium	1			2	3	5
	a	b	c			
Bonus	$312{,}3 \pm 4{,}5$ $n = 605$	$375{,}4 \pm 12{,}1$ $n = 452$	$525{,}8 \pm 33{,}5$ $n = 152$	1662 ± 26 $n = 84$	1118 ± 32 $n = 117$	904 ± 42 $n = 377$
erectoides 23	$455{,}7 \pm 13{,}2$ $n = 438$	$499{,}4 \pm 14{,}1$ $n = 286$	$764{,}2 \pm 22{,}8$ $n = 251$	1979 ± 52 $n = 79$	1248 ± 134 $n = 109$	992 ± 77 $n = 311$
	$t = 9{,}676$ $P \ll 0{,}001$	$t = 6{,}667$ $P \ll 0{,}001$	$t = 58{,}86$ $P \ll 0{,}001$	$t = 5{,}456$ $0{,}02 \gg P > 0{,}01$	$t = 0{,}942$ $P \gg 0{,}2$	$t = 1{,}001$ $P \gg 0{,}2$

Intern. 1 a, b, c: Drei verschiedene Höhen des 1. Internodiums.

Im ersten Internodium sind die Parenchymzellen von *erectoides* 23 um ca. 40 % grösser als bei *Bonus*.

Die übrigen in der Einleitung genannten Eigenschaften als innere Ursache für Standfestigkeit sind nicht näher untersucht. Es ist vielleicht nur darauf hinzuweisen, dass auch die starke Verkürzung der Ähre, sowie ihre aufrechte Stellung in Verbindung mit der Verstärkung der obersten Internodien viel geringere Angriffsmöglichkeit für die das Lagern hervorrufenden äusseren Faktoren bietet.

Die Getreidehalme sind durch Nodien verstärkt und ihre mechanische Bedeutung liegt auf der Hand. Fig. 5, 6 und 7 veranschaulichen die Verhältnisse, wie wir sie im Knoten zwischen dem 1. und 2. Internodium finden. Die anatomischen Veränderungen decken sich ganz mit den in den Internodien gefundenen, ja sie treten ganz besonders klar hier hervor. Die Schnitte in den vergleichbaren Höhen weisen einen grossen Unterschied in der Gesamtquerschnittsfläche auf. Die Lage der Gefässstränge, die Verbindungen der Spross- und Blattspurbündel (Fig. 5) sind in beiden Typen die gleichen. Das Sklerenchym ist vollständig gleich ausgebildet und angeordnet. Der Unterschied besteht in der Grösse der einzelnen Elemente und es ist für alle Gewebe: Sklerenchym, Xylem, Phloem, Parenchym die Zellvergrösserung ersichtlich. Fig. 7 zeigt bei gleicher Ausschnittsfläche und Vergrösserung die Unterschiede im Detail. Die Gefässbündel nehmen in der Mutante einen grösseren Raum ein, bedingt durch die Grösse der Einzelzellen. Die Zellumina im äusseren Sklerenchym sind erweitert. Fig. 6 gibt Aufschluss

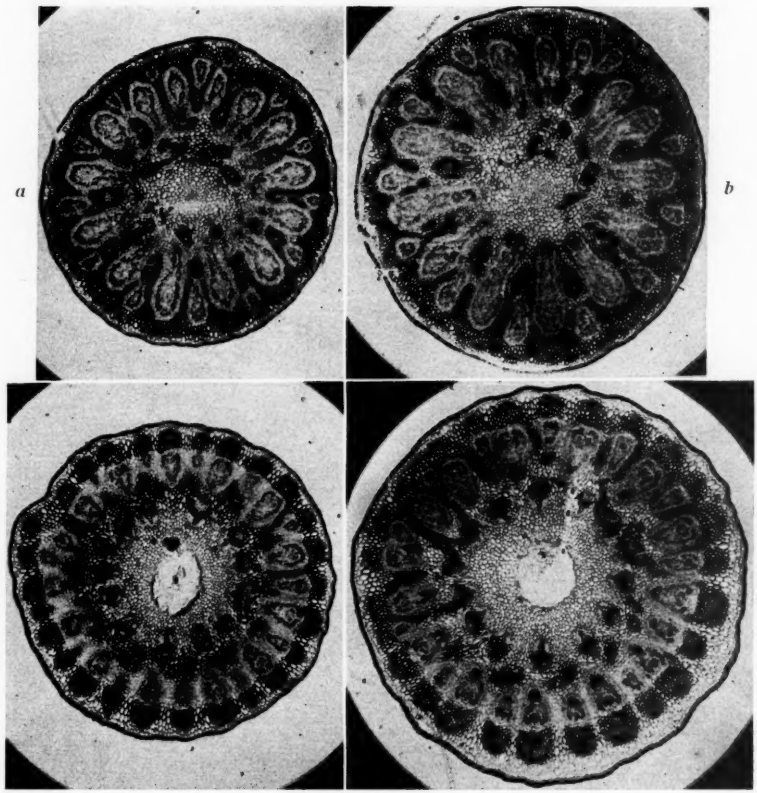
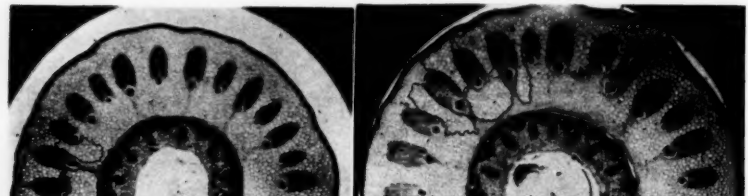


Fig. 5. Querschnitt in der Mitte eines Knoten, *a*: *Bonus*; *b*: *erectoides* 23. Querschnitt durch den gleichen Knoten etwas höher: *c*: *Bonus*; *d*: *erectoides* 23. (Vergr.: *a*, *b*, *c*, *d*: ca. 17×.)

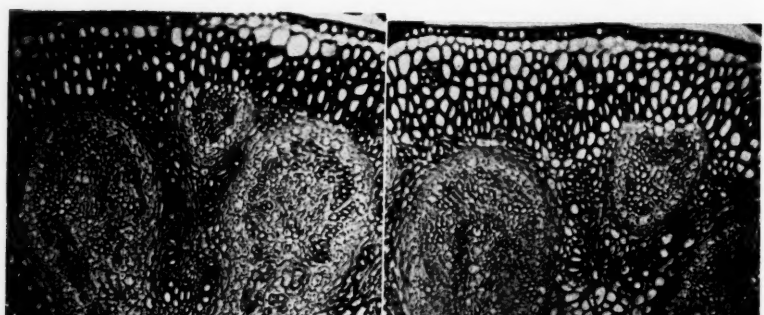
über die Verhältnisse in den Blattscheiden. Ebenfalls die Blattspurbündel zeigen die Vergrösserung, bei konstanter Anzahl. Das Parenchym bietet eine sehr einheitliche Zellgrösse und als Ergänzung seien mittlere Einzelzellflächen für die mit Tusche ausgezeichneten Teile angegeben. Sie sind aus vergleichbaren Stellen zwischen den Bündeln gewählt und wurden bei stärkerer Vergrösserung planimetriert:

Bonus	$1312{,}4 \pm 19{,}7\ \mu^2$	n=394	$t=5{,}058,\ P < 0{,}001$
erectoides	$1441{,}3 \pm 16{,}2\ \mu^2$	n=425	



a *b*

Fig. 6. Oberster Teil des Knoten von Fig. 5. *a*: *Bonus*; *b*: *erectoides* 23. (Vergr.: ca. 17×.)



a *b*

Fig. 7. Detailbilder aus Fig. 5 *a*, *b*. *a*: *Bonus*; *b*: *erectoides* 23. (Vergr.: 78×.)

Auch hier finden wir die Zellvergrösserung. Da die Blattscheide eine gewisse Bedeutung für die Standfestigkeit besitzt, dürfte sich diese Verstärkung günstig auf die Halmfestigkeit auswirken.

DISKUSSION.

Die Beziehungen zwischen Struktur, sowie Aufbau der Halme und Standfestigkeit wurden in allen Getreidearten vielfach studiert. In den meisten Arbeiten wird versucht Sortenunterschiede der Standfestigkeitsbewertung mit sorteneigenen Halmeigenschaften zu vergleichen. Vereinzelt ist die Vererbung der Lagerungsresistenz bedingenden morphologischen Merkmale untersucht (HOWARD, 1912; PLAHN-APPIANI, 1914; BOSE *et al.*, 1937). Bisher nie war die Möglichkeit gegeben morphologische Strukturen als Ursache der Standfestigkeit an Material zu analysieren, das vom Vergleichsmaterial durch einen einzigen Genunterschied getrennt ist. Fassen wir die Ergebnisse der früheren Sortenuntersuchungen zusammen, so zeigt sich, dass Standfestigkeit auf einem Komplex zahlreicher struktureller Halmeigenschaften beruht. In dieser Sorte kann grosse Standfestigkeit durch Ausbildung bestimmter Festigungselemente erreicht werden, in jener Sorte kann die gleiche Standfestigkeit durch stärkere Ausbildung anderer Festigungselemente und veränderter Anordnung derselben erzielt werden. Es gibt nicht ein einzelnes morphologisches Merkmal, das als Mass für die Beurteilung der Standfestigkeit verwertet werden kann.

Kurzstrohige Sorten haben im Allgemeinen grössere Standfestigkeit (RAUM, 1932; ATKINS, 1938). Die gleichen Autoren heben die Bedeutung der Kürze der unteren Internodien für die Standfestigkeit hervor (vergl. auch ROEMER und RUDORF, 1950). Die Querschnittsfläche des Halmes ist grösser bei Sorten mit Lagerungsresistenz (CLARK and WILSON, 1933; ATKINS, 1938). BRADY (1934) weist die Bedeutung der Dicke der Halmwand und des Sklerenchyms nach, und diskutiert die Anzahl der Gefässbündel. Während MOLDENHAUER (zit. nach GARBER und OLSON, 1919) standfesteren Sorten mehr Gefässbündel zuschreibt, können GARBER und OLSON (1919) diese Auffassung nicht unterstützen. BRADY zeigt eine positive Korrelation zwischen Querschnittsfläche und Bündelzahl auf. Die standfesteren Sorten von BRADY, sowie von GARBER und OLSON haben dickere Sklerenchymzellwände. Schliesslich wird das Gewicht per Einheit Strohlänge in Beziehung zur Bruch- und Biegungsfestigkeit gesetzt (ATKINS, 1938). Wenn die einzelnen Autoren nur einzelne aller dieser Eigenschaften als Ursachen für die Sortenunterschiede namhaft machen konnten und sich die Befunde z. T. zu widersprechen schienen, so liegt der Grund in der Mannigfaltigkeit der standfestigkeitsbedingenden morphologischen Ursachen. Auch unsere Ergebnisse bestätigen dies. In *erectoides* gibt es im Vergleich mit *Bonus* Unterschiede in der Pflan-

zenlänge, Länge der basalen Internodien, Querschnittsfläche der oberen Internodien, Dicke der Halmwand, Dicke des Sklerenchymgewebes und Grösse der Gefässbündel, alles Eigenschaften, die bereits früher als Ursachen für Standfestigkeit festgestellt wurden. Andere Merkmale wie z. B. die Zahl der Gefässbündel sind nicht abgeändert, was nicht bedeutet, dass nicht in anderen Sorten, ja vielleicht in anderen *erectoides*-Mutanten auch diese verschieden sind. Schliesslich ist zu beachten, dass Fehlschläge, Beziehungen zwischen Biegungsfestigkeit und morphologischen Strukturen aufzudecken (BRADY, 1934), auf der Untersuchung nur einzelner Internodien beruhen können, da nur die Analyse aller beteiligten Organe ein vollständiges Bild geben kann.

Von besonderem Interesse für unseren Fall ist die Analyse der Nachkommenschaft einer Kreuzung von einer standfesten und einer lagernden Gerstensorte (BOSE *et al.*, 1937). Die beiden Elterntypen unterschieden sich folgendermassen:

standfest	*lagernd*
1. Sklerenchym von der Peripherie entfernt	Skl. nahe der Peripherie
2. Grosser Durchmesser des Sklerench.-ringes	Kleiner Durchmesser d. Skl.-ringes
3. Sklerenchymzellwand: dick	dünn
4. Tangentialer Diameter der Gefässb.: 181 μ	145 μ
5. Radialer Diameter der Gefässb.: 165 μ	127 μ

Die genannten Autoren fanden zudem eine starke Korrelation in der Ausbildung aller dieser Eigenschaften. Die Nachkommen enthielten die beiden Elterntypen sowie zwei intermediäre Typen. BOSE macht 2 Gene für die Ausbildung des Sklerenchyms verantwortlich. Nach den Spaltungszahlen stellt die standfeste Anordnung des Sklerenchyms die doppelrezessive Form dar. Die quantitativen Charaktere (2—5) sind möglicherweise polyfaktoriell bedingt. Vergleichen wir die Unterschiede mit *erectoides* 23, so stimmen unsere Resultate in Punkt 2, 4 und 5 mit denen von BOSE überein, während wir die Eigenschaften 1 und 3 nicht in unserem Material verschieden fanden.

Einen anderen Einblick in die Problemlage gewährt uns die Arbeit von WELTON (1928). Er untersuchte verschieden standfeste Sorten von Hafer und Weizen unter dem Einfluss von Düngung, Beschattung, Tem-

peratur und Aussaatdichte. Unter gleichen Aussenbedingungen wiesen die standfesteren Sorten im Einzelhalm höheren Trockensubstanzgehalt, und grösseren Lignin-, Zellulose- und Polysaccharidgehalt auf. Bei starker Nitratdüngung ist durch grössere Standdichte die Beschattung eine stärkere, was Neigung zu Lagern auch in den standfesten Sorten hervorruft. Die Lagerung wird nach WELTON bedingt durch eine geringere Produktion von Trockensubstanz, Zellulose und Polysacchariden per g Frischgewicht und Einheit Halm. Direkte Beschattungsversuche bestätigten diese Ansicht. Anatomisch ist, parallel zu diesen Befunden, eine grössere Gesamtquerschnittsfläche, die stärkere Verholzung und die Vergrösserung der Parenchymzellen an den Individuen der unbeschatteten und dünngesäten Bestände ersichtlich aus den der Arbeit beigefügten Bildern. Berücksichtigen wir ferner, dass KRAUS (zit. nach KÖNIG, 1928) bei Nitratdüngung eine Verlängerung der unteren Internodien angibt, so gibt dies den Befunden von *erectoides* 23 möglicherweise eine weitere Bedeutung; denn gerade die Gesamtquerschnittsfläche, Zellgrösse und Kürze der basalen Internodien dieser Mutante stellten sich als die auffallendsten Eigenschaften für die günstige Beeinflussung der Standfestigkeit heraus. Die Mutante entwickelt auch bei hoher Nitratdüngung kurze basale Internodien und grosse Zellen, vielleicht als Folge einer geringeren Beschattung, wie für *Bonus* unter diesen Aussenbedingungen im Bestand gegeben ist. Für den Unterschied in der Standfestigkeit zwischen *erectoides* 23 und *Bonus* ohne und bei schwacher Düngung ist demnach eine Genveränderung verantwortlich, das Grösserwerden der Differenz in der Standfestigkeit bei steigender Düngung lässt sich auf die mit der Genmutation verbundene Änderung in der Reaktionsnorm zurückführen. Genaue Untersuchung der Bestandsdichte und Bestockung werden diese Auffassung zu bestätigen haben.

KÖNIG (1928) vergleicht die Morphologie von *nutans* und *erectum* Gersten. Er findet neben der Verkürzung der Ähre und geringerer Pflanzenlänge, kurze basale Internordien für *erectum* charakteristisch. Auch ist in bezug auf Lagerungsresistenz *erectum*-Gerste weniger empfindlich gegenüber N-Düngung. So dürfte also *erectoides* 23 in den Halmeigenschaften der *erectum*-Gerste sehr nahe kommen. Ein bedeutendes systematisches Merkmal von *erectum* ist die Grube an der Kornbasis (GUSTAFSSON, 1941). *Erectoides* 23 besitzt diese Grube nicht, während sie an anderen Mutanten gefunden wurde. Dieses Merkmal muss also nicht mit den übrigen *erectum*-Eigenschaften des Halmes verbunden sein.

Die vorgelegten Befunde unterstreichen die Auffassung GUSTAFSSONS, dass induzierte Mutanten in der Pflanzenzüchtung von grosser Bedeutung sind, zumal hier durch einen Mutationsschritt die Morphologie des Gerstenhalmes grundsätzlich anders geworden; ohne Beeinträchtigung des Ertrages ist ein Halmaufbau entstanden, der auch bei maximaler Düngung Lagerungsresistenz gestattet.

Bei der Frage der Bedeutung der Genmutation in der Evolution ist nach F. v. WETTSTEIN der Einwand, dass die meisten Mutanten mit auffallenden morphologischen Abänderungen vitalitätsgeschwächt und monströs sind, »so alt wie die Deszendenztheorie überhaupt und wird durch die Erfahrungen der experimentellen Mutationsforschung nur gewichtiger». »Zu den bekannten experimentellen Befunden, die diese Einwände abzuschwächen vermögen» (vergl. F. v. WETTSTEIN, 1941) gibt der Halmaufbau von *erectoides* 23 ein neues Beispiel. Durch einen einzigen Mutationsschritt ist die Zellgrösse heraufgesetzt und damit die Morphologie der Internodien, Knoten und Blätter in gleicher Art verändert. Die neue Formbildung der Organe ähnelt stark der einer anderen Varietät der Gerste, gewährleistet extreme Standfestigkeit und gibt dadurch der Mutante unter bestimmten Aussenbedingungen gegenüber *Bonus*-Gerste positiven Selektionswert. Kann eine mutative Abänderung des Halmaufbaues in bezug auf Festigkeit »zweckmässiger» vor Augen treten als in *erectoides* 23?

Weitere Untersuchungen werden Aufklärung zu geben haben über die Verschiedenheiten der Halmmorphologie in der speziellen Allelserie, aus der *erectoides* 23 herausgegriffen ist.

Es ist mir ein ganz besonderes Bedürfnis, Herrn Prof. Dr. ÅKE GUSTAFSSON für Anregung zu dieser Untersuchung und stetige Diskussion meinen ergebensten Dank auszusprechen. Für grosszügige Überlassung von Arbeitsmöglichkeiten in seinem Institut sei Herrn Prof. Dr. A. MÜNTZING bester Dank.

SUMMARY.

A comparative analysis of the morphological and anatomical culm-characters in relation to lodging was carried out in *Bonus* barley and its extreme straw-stiff mutant *erectoides* 23. The elasticity against bending was determined for all internodes separately. The upper internodes of *erectoides* 23 are significantly more resistant to bending compared with

Bonus. There is no difference in the lower internodes. The morphological investigation showed that the increased straw-stiffness of *erectoides* 23 is partially due to the decreased total length of the culm and the shorter basal internodes. The high resistance to bending in the upper internodes could be shown to be correlated with a larger total cross section area and a bigger pith cavity. The anatomical analysis revealed no difference in the basal internodes, while the upper internodes show a stronger developed sclerenchym and a larger cross section area of the vascular boundles as determined by measurement of the tangential and the radial diameter. The nodes, as well as the culm enclosing leaves, showed the same changes in their structure as found in the internodes. The cells of the sclerenchymatic tissue, the vascular boundles and the parenchym in all organs of the upper culm-part appeared to be larger. It is suggested that the increased cell-size is the cause of the anatomical changes influencing straw-stiffness in *erectoides*. The findings are discussed in relation to the literature.

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Communications from the Swedish Seed Association. No. 62.

CHONDRILLA CHONDRILLOIDES, A NEW SEXUAL CHONDRILLA SPECIES

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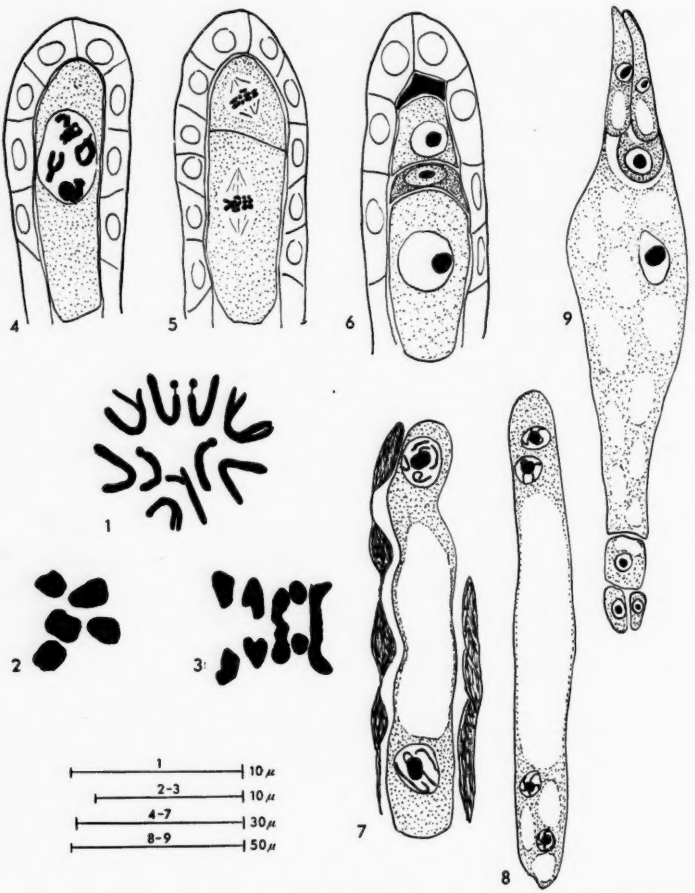
THE genus *Chondrilla* includes, at present, about thirty described species which mainly have an Asiatic distribution. Only a few representatives occur in Europe, among which *Ch. juncea* is well-known as being one of the first cases of agamospermy (ROSENBERG, 1912). On examining nine other *Chondrilla* species, PODDUBNAJA-ARNOLDI (1933) could establish agamospermy in eight of them, only one proving to be sexual, viz. *Ch. ambigua*. As to the chromosome numbers, all agamosperms had $2n=15$ or 20. *Ch. ambigua* alone had $2n=10$.

The present author has long been interested in the genus *Chondrilla* but so far has only dealt with *Ch. juncea*. Last year, I had the opportunity of collecting material of *Ch. chondrilloides* F. et M. raised from seeds procured from the Botanical Garden in Graz, Austria. As root tip mitoses revealed only 10 chromosomes, there was reason to suspect a new case of sexual reproduction within the genus. This could also be confirmed by a cytological investigation, the result of which is published in the present paper.

Ch. chondrilloides F. et M. is synonymous with *Ch. prenanthoides* VILL. The latter name is used by the Russian expert Professor M. M. ILJIN (1930) who places it in a special section, *Lagoseropsis* ILJIN, together with two other species, *Ch. Mattfeldii* STOJ. et STEFF. and *Ch. Urumoffii* DEG. *Lagoseropsis* is the only section which is not represented in the Soviet Union; its distribution is mainly in south-east Europe. *Ch. condrilloides* occurs in the mountains of east Middle Europe and south-east Europe.

Somatic chromosomes. — As already mentioned, *Ch. chondrilloides* has a somatic chromosome number of $2n=10$. Fig. 1 illustrates a root tip mitosis with ten chromosomes at metaphase. As in *Ch. ambigua* (PODDUBNAJA-ARNOLDI, 1933) and *Ch. juncea* (BERGMAN, 1952), two size-groups can be distinguished, one with six long chromosomes and one with four short ones. The size difference between the two chromosome groups is, however, not so marked as in the two other *Chondrilla*



Figs. 1—9. *Chondrilla chondrilloides.* — 1, root tip mitosis. — 2, I-M of PMC in polar view. — 3, I-M of PMC in side view. — 4, ovule with EMC. — 5, ovule with dyad. — 6, ovule with tetrad. — 7, bi-nucleate ES. — 8, four-nucleate ES. — 9, mature ES.

species. Like these, *Ch. chondrilloides* has two long chromosomes with satellites. Compared with *Ch. juncea*, the chromosomes of *Ch. chondrilloides* are somewhat larger.

Sporogenesis. — Microsporogenesis is perfectly normal in this species. Five bivalents are always formed (Figs. 2—3) and the two

meiotic divisions are carried through without any disturbances. The mature pollen grains seem altogether normal.

In the young ovule, one single EMC is always present (Fig. 4). This divides twice, each division being followed by wall formation (Figs. 5—6). Of the four macrospores, the basal one forms the ES (Fig. 6) by three successive divisions. Fig. 7 shows the bi-nucleate ES, Fig. 8 the four-nucleate stage, and Fig. 9 the mature ES. Consequently, in *Ch. chondrilloides* ES-development is of the monosporic type and results in an eight-nucleate bipolar ES. In these respects, the species corresponds altogether with *Ch. ambigua,* the similarity extending also to the appearance of the antipodes. Like *Ch. ambigua, Ch. chondrolloides* has three antipodes (Fig. 9), which are, however, of a very short duration.

Embryo- and endosperm-formation follow in a normal way after fertilization and castrated heads have given no achenes.

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ABSTRACTS

I. E. GLUSHCHENKO: Correction of a correction. Reply to Dr. ALBERT LEVAN and Professor ARNE MÜNTZING.

Strange things do happen in the world. And what I'm going to tell about is one of them. In July, 1950, the VII International Botanical Congress was held in Stockholm. Soviet botanists were invited to take part in the work of the Congress. I happened to be one of the Soviet delegates. At the genetics section I read my paper on »The Hybridization of Plants by Means of Grafting». Like many other delegates of the Congress, I became acquainted with the work of a number of botanical, genetic and plant breeding institutions in the country.

On returning from Sweden I wrote about my impressions in the »Literaturnaya Gazeta» (No. 72 [2663], August 22nd, 1950). As I later learned, a part of my report was translated into German and published in the »Tägliche Rundschau» on September 15th, 1950.

In my report I mentioned, in particular, that certain scientists (for instance, the President of the Congress, Prof. SKOTTSBERG) tried to misrepresent the true state of botanical science in certain countries. I referred to his undue and unmerited praise of the state of botany in the United States of America. I pointed out that, judging from the reports of the leading American botanists, and of Professor BLAKESLEE in particular, Professor SKOTTSBERG's evaluation was, to put it mildly, much too high.

Being a plant geneticist, I was mainly interested in the papers in my own line, and actively attended the meetings. At the same time from the theses of the papers that were read at the other sections and from the impressions of my colleagues who attended the meetings, I was able to form my opinion about them as well. The impression that I and my colleagues gained from the works presented at the Congress was that nothing fundamentally new in the theory of botanical science was to be found in the reports of the foreign scientists. The branch of science which claims to be the theoretical foundation of biology, namely Morganism, remains just as it was — fallacious in theory and sterile in practice. At the Congress my former opinion of Morganism was still further strengthened.

In my report I wrote that although the Soviet delegation was small, it nevertheless became the centre of attention. What proof is there of this? In the first place, the articles that appeared in the Swedish press; and secondly, the interest manifested by the delegates in the papers we read. For instance, the Social-Democratic paper »Morgontidningen» wrote on July 18th that during the lectures of the Soviet delegates »the auditorium was filled to the last place and some had to sit on the floor . . . ».

The numerous questions and especially the talks with the scientists of different countries after the lectures showed the deep interest of the scientists in Michurin biology. I should like to inform Dr. LEVAN and Professor MÜNTZING that after we read our papers we found in our mail box at the Congress a number of letters thanking us for our lectures. The following letter may serve as an example:

»Dear Prof. Glushchenko,

I am writing to thank you for the interesting papers you and your colleagues read the day before yesterday. It is a pleasure to see you here and I hope we shall see you at the future congresses and conferences. I think that your translater deserves especial praise. She managed her difficult task very well.

Sincerely yours,
Peter Bell».

Unfortunately I am not acquainted with Peter Bell.

But I have somewhat digressed from the subject. Let us return to the point. I concluded my report with some special remarks about the Swedish scientists MÜNTZING and LEVAN and about the institutions which they courteously showed us. I wrote about the good impression that I gained from our meetings, Professor MÜNTZING and Dr. LEVAN, and about the interest you manifested in discussing scientific problems during our extended talks. I took that to be a sign of your desire to understand Soviet scientists, to discuss scientific problems and our personal experiments.

I can express an equally favourable opinion of the plant breeding work in Svalöf and Landskrona conducted along classical lines (i. e. by means of crossing and selection applied long before the work of MENDEL had been rediscovered).

What displeased me and my colleagues? I did not conceal it then and I shall not deny it now that it was Morganism »in action», it was the practical results of Morganism that we did not like. Neither your polyploid rye, Professor MÜNTZING, nor Prof. GUSTAFSSON's X-ray-induced mutations in barley could convince us that polyploidy and X-ray-induced mutations are a promising path in plant breeding. Neither on experimental plots nor in written papers can proof be found in favour of polyploidy.

That is the gist of my report published in the »Literaturnaya Gazeta» (Moscow) in 1950. More than a year later a »Correction of a Report» by Dr. ALBERT LEVAN and Prof. ARNE MÜNTZING appeared in the journal »Hereditas» (Vol. XXXVII, 1951).

I was extremely surprised by this article and especially by the tone and style in which it was written. I could hardly believe that it came from the pens of Dr. LEVAN and Prof. MÜNTZING.

The authors of the article write that I had no right to pass judgment on the numerous papers that were read at 15 different sections which to a great extent were working simultaneously, since I couldn't attend all of them. But as has been already mentioned, the theses of all the papers were available to everyone. I, personally, attended the section of genetics and cytology while my colleagues attended the sections of agronomical botany, forest botany, plant anatomy and morphology, plant physiology, phytopathology and phytogeography, i. e. the chief botanical sections. Naturally, I could form an opinion of the entire work of the Congress and communicate my impressions to my readers.

As to Prof. SKOTTSBERG's high estimation of botany in the New World, I must say that Prof. MÜNTZING and Dr. LEVAN have strengthened my objection to it. They once more refer to BLAKESLEE's paper and maintain that his works »have always been characterized by their immediate bearing on fundamental biological problems». Yet, in our opinion, BLAKESLEE's works have no direct relation to biological problems, let alone fundamental ones; since they are of absolutely no practical significance either, we must regard the interest manifested by some foreign biologists in these works as nothing but lackey servility to Uncle Sam.

But in connection with my judgment of BLAKESLEE's works Dr. LEVAN and Prof. MÜNTZING found it necessary to write the following: »The statement of Dr. GLUSHCHENKO is interesting, however, because it shows him to have little appreciation of research work that does not immediately aim at practical application». Let me remark, Dr. LEVAN and Prof. MÜNTZING, that the most profound theoretical researches in biology are being conducted precisely in the Soviet Union. In particular, the author of this article has been working for a long time on the genesis of the plant cell. It is precisely Soviet science that has made such outstanding theoretical discoveries in biology as the theory of phasic development of plants, the theory of vitality, the theory of the origin of cells from non-cellular living substance, etc. As far as BLAKESLEE's works are concerned, their fault lies not in the fact that they are highly theoretical and do not aim at immediate practical application. The thing is that they are conducted on the fallacious theoretical foundation of Morganism, in other words, that they are void of theory.

Dr. LEVAN and Prof. MÜNTZING are annoyed with the fact that I wrote about the interest the participants of the Congress manifested in the lectures of the Soviet scientists. »It is correct that the special meeting in the GEN section was attended by a large audience», my opponents confirm. But according to them the main reason for this was that »the majority of the listeners, gathered at this meeting, had come not so much in the hope of learning new scientific facts or theories but in order to see in person and to listen to people who deny even the most elementary facts of the science of genetics. Chemists, denying the existence of molecules or atoms, would undoubtedly attract an equally numerous audience, were they allowed to ventilate their opinions at an international chemical congress».

What can be said in reply to this scandalously tactless statement?

Firstly. The Soviet delegates never thought that they might serve as a source of entertainment for the learned gentlemen. Moreover, we did not feel this to be so in Sweden either before or after our lectures. Had the Soviet delegates felt this, they would have immediately left the Congress and would have been perfectly justified, because they had come to a scientific meeting and not to a theatrical performance. In fact, I am sure that LEVAN and MÜNTZING themselves do not believe what they write. I have a number of reasons for thinking so, and one of them is this. Quite recently I visited Denmark and gave a lecture on the genesis of the plant cell at the University of Copenhagen. To my surprise I saw Prof. MÜNTZING in the audience. As I learned from my talk with him he had come from Sweden for the sole purpose of attending my

lecture. Indeed, Prof. MÜNTZING insisted on my coming to Lund (Sweden), where he conducts his work, to repeat my lecture.

Secondly. Michurinists have never denied a single fact of Morganist genetics, if it actually was a fact. We differ, Dr. LEVAN and Prof. MÜNTZING, in another point, namely in the estimation and the interpretation of the facts. Our interpretation fundamentally differs from yours. You accuse me several times of denying, as all other Michurinists do, the role played by chromosomes in heredity. This is a failure to understand the Michurin point of view on heredity. Michurinists cannot deny the existence of chromosomes (because they are a fact), nor can they deny that chromosomes do play a role in heredity. One of the fundamental precepts of Michurin genetics is that every living particle, molecule, or granule of the living body possesses the property of heredity; it therefore follows that no exception is made for chromosomes. Thus my opponents are forcing an open door as regards this question.

What do Michurinists deny? I shall answer frankly: they deny the chromosome theory of heredity, viz. the attempt to regard the chromosomes as a special organ of heredity. It is this that we consider to be the greatest error of some of the twentieth century biologists. This theoretical error leads to sterility in practice, a point which will be discussed more fully later on.

Thirdly. To draw a comparison between the molecular theory in chemistry with the chromosome theory of heredity is tantamount to comparing the actually existing elephant with the mythical phoenix. Chemistry is based on a thoroughly sound general theory, and we see what gigantic steps this science has been making and how it enriches knowledge, practice and mankind. Weismannist-Morganist genetics is based on the speculative theory of the gene, and we see that during the fifty years of its existence this »science» has remained sterile.

I can console my opponents by the fact that the idea of the practical sterility of Morganism is entertained by their like-minded friends as well.

»The progress of genetics», writes Prof. MATHER, »has not yet led to the marked advances in plant and animal breeding which has been so confidently expected in the past». (»Nature», 1944, 153, No. 3895.)

»Genetics owes a debt to plant and animal breeding both for its foundation and its development», writes DARLINGTON. »If the purpose of agriculture in the future is to be the highest production, genetics will have the opportunity of repaying this debt.» (»Nature», 1944, 153, No. 3895.)

Has Morganist genetics repaid this debt today?

Here I approach the main thesis of my opponents. It is evidently this point that induced them to publish a correction of my report in »Hereditas».

The authors of the »Correction» write:

»Dr. GLUSHCHENKO may recall that during his visit to Svalöf after the Congress the present writers had the pleasure of directing his attention to x-ray-induced mutations in barley and in other plants, as well as to colchicine-induced tetraploids of, among other things, red clover, alsike clover and rye. These new types are perfectly viable and have an undeniable practical significance. Within red clover, alsike clover and rye, several tetraploid types are at present propagated on a large scale. These types will be released for

practical agriculture in the near future, the tetraploid rye already in this year. Thus it can be stated that after about twenty years of experimental work with the artificial production of mutants and polyploids the first practical results are now appearing. This is a good illustration of the fact that the new breeding methods, viz. mutation breeding and polyploidy breeding, are gradually taking their position besides the classical breeding methods.»

It is true that I and the other Soviet delegates saw in Svalöf GUSTAFSSON's X-ray-induced mutations in barley and also MÜNTZING's colchicine-induced polyploid rye and LEVAN's polyploid clover. But the point is that we were not enraptured with what we saw.

Why? Let us answer this question.

Prof. GUSTAFSSON showed us the variety tests of the barley. At that time there were 60 X-ray-induced mutations. As Prof. GUSTAFSSON himself said, 54 of them were less productive than ordinary barley and only six were by 5 to 10 per cent more productive. Every plant breeder knows that such results are of no practical significance.

According to Dr. LEVAN the polyploid clover produces more green mass, but gives by 25 per cent less seed than ordinary clover.

As to Prof. MÜNTZING's polyploid rye, not only its author but other Morganists, too, have evidently set great hopes on it. The fate of their theoretical expectations depends upon it; it is believed that with the doubling of the set of chromosomes the yield of the rye plant will be greatly increased. Let me for a moment digress from my own impressions and refer to the materials that appeared in print in Sweden concerning this rye.

I have before me Prof. MÜNTZING's paper »Cyto-genetic Properties and Practical Value of Tetraploid Rye». (Hereditas, 1951, Vol. 37, Nos. 1—2.) From the author's summary we learn that the number of flowers per spike in the tetraploid rye is lower than in the ordinary plant, the seed setting in ordinary rye is by 20—25 per cent higher than in the tetraploid form, besides the tetraploids show a tendency to shed basal spikelets before maturity.

»Kernel yield» writes the author, »has been studied for 8 years and in six different places in South and Central Sweden ... *Data obtained from 18 different trials failed to reveal any significant difference in yield between diploid and tetraploid Steel rye.*» The conclusions are far from optimistic, indeed. True, the author writes that the rye is propagated chiefly due to its good baking properties. But what has that to do with tetraploidy and where is the expected sharp rise in yields?

Still less optimistic are the results of R. SCHILDT and E. ÅKERBERG from the comparative tests of tetraploid and diploid rye that had been conducted in 1949 at the Ultuna branch of the Swedish Seed Association. They were published in »Sveriges Utsädesförenings Tidskrift».

It seems that the rye with which Prof. MÜNTZING began to work as far back as 1930 has been undergoing tests here since 1945 to determine its yield, its tillering, flowering and seed setting. The results of the tests are as follows:

The tillering (the formation of fruiting shoots) was by 7—8 per cent lower in the tetraploids than in the diploid rye.

The total average number of flowers per spike including the undeveloped flowers was by about 10 per cent lower in the tetraploid than in the diploid forms. The spikes of the tetraploids were, as a rule, smaller than those of the diploids.

The number of undeveloped flowers in ordinary rye amounted to 14,8 per cent, while in the tetraploids it was 22,9 per cent.

In the ordinary rye the number of flowers with well-developed kernels was by 12—15 per cent higher than in the tetraploids. True, the average weight of 1000 grains of the tetraploid rye was by 44 per cent higher than of the diploid, but the *kernel yield in tetraploids was 12 per cent lower than in the diploid rye.* (»Sveriges Utsädesförenings Tidskrift», 1951, Vol. 61, No. 5, pp. 240—263.)

Such is the factual side of the problem on which »the attention of the world's cytologists and geneticists is centered today» (LEVAN and MÜNTZING).

So things stand, approximately, with the theory of inbreeding (Inzucht) which »is not accepted in Soviet Russia as an instrument in practical breeding». In this connection our opponents express their regret that »the Soviet state will suffer immense economic losses on account of this dogma».

The authors write: »The great increase in yield attained by the so-called double-cross method which has during recent years led to outstanding practical results in the USA, above all within maize, will be lost to Soviet agriculture if the inbreeding of cross-fertilizers is not permitted».

The authors are strangely concerned with the fate of Soviet agriculture. It would seem they should be more concerned with the fate of their own national agriculture since it is nearer to them and they come in direct contact with its requirements. Why is it then that inbreeding is not applied in the practical farming of Sweden in the cultivation of winter rye, one of the country's chief cultures? Why do they cite examples from practical farming in the USA? The answer is quite simple. Swedish scientists have been working for many years on the application of inbreeding to rye. And with what results? No one can answer this question better than those who have been working in this field and who adhered to the theory of inbreeding in their time.

An excellent document in this respect is the paper of Professor HERIBERT NILSSON: »Eine Prüfung der Wege und Theorien der Inzucht» (»Hereditas», 1937, Vol. 23, Nos. 1—2). He wrote: »By means of isolated breeding a sharp differentiation of the gene material can, therefore, be attained both in allogamous and in autogamous plants. Lines can be created with firm stems, or with short stems, or green kernels and even with a potential high productivity, but all of them are depressed forms. It would be desirable to be able to restore the viability of the forms possessing these properties by crossing such lines. But this cannot be done, at any rate not in the case of rye. My manifold endeavors to take this course in breeding have ended in *utter failure.* There is no necessity to stress the fact again that in this respect I have not been biased, yet the results of my successful and continuous experiments force me to give up the theory of heterosis (from which I proceeded since I considered the explanations it offered to be very probable) as the basis of work in plant breeding».

NILSSON wrote further: »In theoretical investigations one can persistently try to prolong the life of a pet theory since it often does no harm and calls forth discussion and checking. *But in practical breeding it is absurd to conduct work on the basis of a theory which results in the appearance of varieties that are less productive than the parental one*».

One cannot but agree with this.

Hybrid maize has nothing to do with the theory of inbreeding as presented by the Morganists. Indeed, it actually contradicts the theory of Morganism.

Here is what the well-known Morganist R. A. FISHER has to say about it:

»It is characteristic of the great work of maize improvement that it is very largely empirical, and without any distinct or satisfying analytic basis. Abundant enterprise and strong public support are its mainsprings; theoretical ideas have, on the whole, been insufficient to explain what has been achieved». (R. A. FISHER, British Science News, 1949, 1 (10).)

I can assure our opponents that the theory and the practical aspect of the benefits of heterozygosity for field cultures, including the improvement of cross-fertilizers, has been developed for many years now in the Soviet Union. The first who raised this problem and found its practical solution was the outstanding Soviet scientist Academician T. D. LYSENKO. The results of this work are now broadly applied on Soviet collective farms, and this has led to a considerable increment in the annual yields. Dr. LEVAN and Prof. MÜNTZING should know about that.

My opponents have taken offense at the idea of my having hinted that they are Michurinists. I cannot conceive where LEVAN and MÜNTZING found such a »hint». I made no such statement in the report that appeared in the »Literaturnaya Gazeta» and was later reprinted in the »Tägliche Rundschau», since there were and are no grounds for it as far as they are concerned.

What I did write was that »differentiation is taking place among formal geneticists abroad» and that »the reactionary scientists who faithfully serve imperialism and slander Soviet science cannot be identified with the mass of Western botanists». This statement I wholly confirm today. With every year, we, Soviet biologists, become convinced that the number of our friends abroad is constantly growing. Nor is this friendship purely platonic. Our friends write books and articles on the subject. I can refer to the works of the English biologists JAMES FYFE (»Lysenko Is Right») and ALAN MORTON (»Soviet Genetics»). I urgently advise Dr. LEVAN and Prof. MÜNTZING to acquaint themselves with these works and also to study the original works of Soviet biologists.

I assure you, gentlemen, there will then be less of the idle talk, misunderstanding and confused ideas about us with which your strange and rather puzzling article is so amply sprinkled.

20.III.1952.

ALBERT LEVAN and ARNE MÜNTZING: Comments on Professor GLUSHCHENKO's »Correction of a correction».

We have had the opportunity of seeing the manuscript of Professor GLUSHCHENKO's reply to our »Correction of a report» published in this journal last year. We appreciate his readiness to continue our discussion about the VIIth International Botanical Congress in particular and genetic problems in general. Though the present contribution by Prof. G. is more carefully written and less burdened by aggressive propaganda than his previous article, there still remain several points which, in our opinion, should not be left unanswered.

In his previous article Prof. G. wrote that »the members of the congress were offered more than 500 papers among which only few had any scientific interest». On account of our criticism of this very sweeping judgment Prof. G. has now explained that his meaning was that »nothing fundamentally new in the theory of botanical science was to be found in the reports of the foreign scientists». This is, indeed, something quite different from the previous statement, and it is certainly rather rare that »fundamentally new discoveries» are presented at international congresses. We do not feel competent to judge whether such discoveries were reported at the Botanical Congress in question, but we are sure that most of the papers presented had scientific interest and reflected recent progress in the many different branches of botany.

In both his articles Prof. G. stresses the fact that the geneticists in the Soviet delegation attracted a large audience during a special session devoted to their papers. This is considered to demonstrate the superiority of »Michurin genetics» over »Mendel—Morgan genetics». Although it was distasteful to us we had to inform Prof. G. that the majority of the listeners gathered at this particular meeting not so much in the hope of learning new scientific facts or theories but rather in order to see in person and listen to people who deny even the most elementary facts of the science of genetics. As this is still perfectly true, we do not find it possible to change or modify the statements concerning this event made in our previous article (pp. 297—298). Some non-genetical lectures were given by other members of the Soviet delegation. We have been told that these lectures were excellent and of a high scientific quality.

In his present article Prof. G. formulates his opinion of genetics in the following way: »The branch of science which claims to be the theoretical foundation of biology, namely Morganism, remains just as it was — fallacious in theory and sterile in practice». We do not think that »Morganism» or »Weismann—Mendel—Morgan-genetics» has ever claimed to be the theoretical foundation of biology, but we find it undeniable that genetics has been highly stimulating to the development of biological research in this century, and it may even be said that this science now occupies a central position in biology. In a recent review GOLDSCHMIDT (1950) has dealt with this subject. May we recommend Prof. G. to study this review carefully? We would also appreciate it if Prof. G. could devote some time to a study of the contributions made by BLAKESLEE and his collaborators to the development of cytogenetics. Prof. G. holds now the opinion that »BLAKESLEE's works have no direct relation to biological problems, let alone fundamental ones». Such a

statement can only be made by persons who have not read or understood the very important contributions made by BLAKESLEE and his collaborators, especially BELLING. Every geneticist knows about their classical studies on trisomics, haploids and tetraploids in *Datura*, and about the discovery of reciprocal translocations and their effects in this material. By such studies it was possible to demonstrate not only that the different chromosomes of the *Datura* genome have specific effects but also that each chromosome is differentiated into segments which influence vigour, fertility, pollen tube growth, *etc.* in a highly specific way.

Before his work with *Datura*, BLAKESLEE made fundamental discoveries concerning sex differentiation in fungi, and later on, in 1937, he became mainly responsible for the introduction of the colchicine method of chromosome doubling. Among BLAKESLEE's research in recent years his successful cultures of weak hybrid embryos should especially be mentioned. By such work many hybrids have been realized which earlier could not be produced at all. Do you really maintain, Prof. G., that such work has no direct connection with biological problems? If this be the case, we are afraid that the language we speak is so different that an understanding between us will be impossible.

By the way, don't you ever get tired of spicing your scientific papers with propaganda phrases? In our previous article we wrote the following: »Even at the risk of being called »lackeys of Wall Street» or something else of equal spirit, we wish to point out that the researches of BLAKESLEE have always been characterized by their immediate bearing on fundamental biological problems». This time you did not call us lackeys of Wall Street but used another variant of the old theme: »lackey servility to Uncle Sam» (p. 372).

Your dislike of BLAKESLEE's *Datura* work is probably mainly caused by the fact that this work in a brilliant way confirms what MORGAN and his collaborators had found in *Drosophila*, namely, that the chromosomes are the most important agents of heredity and that changes in the chromosome complement, as a rule, have highly specific consequences. As you should know, even very small sections of a chromosome are, as a rule, indispensable for the life of the organism. True, there may be other cell constituents of great importance, but as yet rather little is known about them in comparison to what we know of the chromosomes. Many facts, indeed, prove that the chromosomes have quite a predominating rôle in heredity.

One such fact was mentioned in our previous article (p. 296), viz., the constancy of allopolyploids. In your present article you have not answered our previous questions on this point. May we remind you that we wanted to know how the phenomenon of polyploidy can be compatible with the Michurin doctrine? Especially we wanted to know whether the Michurin doctrine is able to give a better interpretation than the chromosome theory of the fact that primary species hybrids, when partially fertile, will give rise to a great variation in their progeny, while, on the other hand, after chromosome doubling, as in *Triticale*, the new products immediately acquire constancy and fertility?

Now, we realize that you don't like to talk about polyploidy also for other reasons, and that you are doing your best to prove that our induced polyploids in rye and clover are worthless from the practical point of view. Professor G.

writes: »According to Dr. LEVAN the polyploid clover produces more green mass, but gives by 25 % less seed than ordinary clover». The two artificially doubled clover species, red clover and alsike clover, behave differently in the respect of seed-setting. Whereas alsike clover both in our material and in that of FRANDSEN has already from the start given a seed yield of about the same size as the diploid alsike, the red clover caused considerable trouble on that point. The first tetraploid red clovers were produced in 1940, and their seed yield was decidedly inferior to that normally obtained on diploid red clover. Thus, in certain experiment series at Svalöf the seed yield varied in 1944 from 35 to 133 kg/har, and in 1946 from 67 to 143 kg/har, while the diploid seed yield from the same part of the country may be as high as 300 kg/har (this yield is obtained only under good conditions).

Methodically conducted breeding work for increased seed yield proved, however, to be unexpectedly successful in red clover. According to JULÉN, who is now responsible for the continued breeding and propagation of the tetraploid red clover material once produced by LEVAN, the seed yield of the tetraploids has improved so much as to make them practically equal to the diploids in this respect.

As an instance of this the following figures may be cited (JULÉN, oral communication). One material of tetraploid red clover from the Swedish strains Merkur × Wambåsa (called Svalöf 034) showed the following increase in seed yield from C_4 (the fourth generation after the colchicine-doubling) to C_6:

Year	Generation after doubling	Seed yield kg/har
1946	C_4	30
1948	C_5	190
1950	C_6	316

The last value is calculated from a rather large culture (1236 kgs harvested on 4 hars). The same year the diploid red clover experiments at the Svalöf Institute gave an average yield of 300 kg/har.

Evidently seed-setting is a quality very much influenced by the genotypical balance of the plant. It is thus in perfect agreement with the opinion of both of us, often expressed in our papers on artificial polyploidy, that the newly produced tetraploids, because of their upset balance, should suffer from certain weaknesses, such as disturbances of the gametogenesis, the fertilization, and the early embryo development, all of these matters resulting in a lowered seed-setting. As the genotypical balance is gradually improved by intentional breeding or by automatic mass selection, the raw polyploids begin to behave more regularly in the above-mentioned respects with the result that the seed-setting is increased. How far the improvement of a new polyploid type may be taken can only be shown by experience.

Thus, in the case of red clover the breeding of the tetraploids has succeeded in producing strains which have as good seed yield as the diploids, and which at the same time have a decided increase in green matter yield. The latter increase, which often is as high as 30 or 40 % has, according to JULÉN, for a long sequence of years and in trials all over Sweden given an

average of 10 % increase in yield. Consequently, there is by now no doubt that the polyploidy breeding has given positive results for practical agriculture in this case.

You state that »it is believed that with the doubling of the set of chromosomes the yield of the rye plant will be greatly increased». We must ask you, who holds this belief and why do you talk about »the expected sharp rise in yields»? It is true that in the early days of research on induced polyploidy some people were over-optimistic about the new possibilities, but we do not think you can find any statements of this kind in our papers on polyploidy. On the contrary we realize the great difficulties involved, because we know, thanks to BELLING, BLAKESLEE and others, that the changed mode of chromosome association at meiosis in such autotetraploids will lead to a reduction in fertility and to the production of a certain proportion of unbalanced aneuploids in the offspring. We know, primarily thanks to BLAKESLEE, that such aneuploids have a reduced vigour and fertility. In tetraploid rye the average frequency of such aneuploids amounts to 23 per cent. In spite of this handicap and in spite of some other undesirable features (reduced degree of tillering, *etc.*) the tetraploid rye has a satisfactory yield because the very marked increase in kernel size (about 50 per cent) compensates the other factors which tend to reduce the yield.

As a proof of the inferiority of tetraploid rye Prof. G. cites the results of SCHILDT and ÅKERBERG (1951), who performed a detailed investigation of tetraploid Steelrye at the Ultuna Branch Station of the Swedish Seed Association. Prof. G. writes that the rye in question seems to have been tested at this station since 1945 and that kernel yield in the tetraploids was 12 per cent lower than in diploid rye. This is a misleading statement as the figure in question only refers to the yield in 1949. SCHILDT and ÅKERBERG also point out (likewise in the English summary) that the percentage figures mentioned by them come from the result of a study of a limited material and must not be generalized. As is pointed out by MÜNTZING (1950), the relative yield values of diploid and tetraploid rye show rather great fluctuations owing to the necessity of making indirect comparisons with the help of wheat standards. Only after a relatively long period of testing can something definite be said about the yield of the tetraploid. So far, however, it has behaved rather well. In the summer of 1951 this tetraploid rye was included in a large number of official yield trials in South Sweden and Denmark, the absolute yields ranging from 14,3 to 53,8 deciton per hectare. In 8 of these trials figures are available for both diploid and tetraploid Steelrye. We regret to inform you, Prof. G., that in these trials the average yield of the tetraploid was 8,3 per cent higher than in the ordinary diploid Steelrye. The best results were obtained in the southernmost provinces and in Denmark. At Ultuna, where SCHILDT and ÅKERBERG made their investigation, the tetraploid Steelrye is probably too far to the north to give optimal results.

Prof. G. does not believe that the good baking properties of the tetraploid rye have anything to do with its chromosome number. This is a mistake. The increased kernel size is a result of the increased cell size, and the increase in cell size a consequence of the doubling of the chromosome number. Now, as the volume increases more than the surface when the total size of the

seed is increased, the interior part of the seed will be more predominating and will give a better flour in tetraploid rye than in diploid rye. That big kernels give better baking results than small kernels has already been observed by the bread factories. For that reason they sift the kernels of ordinary rye in order to augment the proportion of big kernels. Quite new baking possibilities have now been obtained after the introduction of tetraploid rye, and a quality premium proportional to the 1000-grain weight will probably be introduced.

After criticizing the practical value of tetraploid rye Prof. G. writes: »Such is the factual side of the problem on which »the attention of the world's cytologists and geneticists is centered to day» (LEVAN and MÜNTZING)». This is again, to put it mildly, a highly misleading quotation. We have certainly not said that about the work on tetraploid rye. The passage in question runs as follows (LEVAN and MÜNTZING, 1951, p. 294): »Among approximately 70 papers of the GEN-section only four had any reference to colchicine-induced polyploids. That many papers both of the CYT- and the GEN-sections were devoted to the experimental induction of changes of the genetic constitution is, in our opinion, only natural. It is on such problems that the attention of the world's cytologists and geneticists is centered today. That these problems are of fundamental interest to the whole of biology is a fact which can hardly be disputed by anyone».

What we had in mind is obviously, in the first place, the induction of mutations. During his visit to Svalöf Prof. G. had the opportunity of seeing Professor GUSTAFSSON's induced mutations in barley. About this material we read the following: »54 of them were less productive than ordinary barley and only six were by 5 to 10 per cent more productive. Every plant breeder knows that such results are of no practical significance». In Sweden, as in most other countries in which new varieties are tested in a reliable way, an increase in yield of 5 to 10 per cent is, indeed, considered to represent a very important step forward, and if you only obtain one or a few such improved products, it does not matter that the great majority of them are inferior. Knowing the effect of the radiation on the chromosomes this is precisely the result to be expected. We also know, however, that after crosses and Mendelian recombination the mutant genes will probably become more valuable than in the primary products of irradiation.

We realize, indeed, very well the difficulties involved in the effects of utilizing induced mutations and induced polyploidy in plant-breeding work, but we have nevertheless very good reasons to maintain that such new methods are gradually taking their position beside the older breeding methods.

Prof. G. tries to prove that during its fifty years of existence »Weismannist —Morganist genetics» has not only been »fallacious in theory» but also »sterile in practice». In support of that he also quotes some sentences written by MATHER and DARLINGTON (p. 373). DARLINGTON writes that »genetics owes a debt to plant and animal breeding both for its foundation and its development», and »if the purpose of agriculture in the future is to be the highest production, genetics will have the opportunity of repaying this debt».

Although DARLINGTON may not be your favourite author, we would recommend you, Prof. G., to study another paper by him, namely »Production genetics in Sweden» (Nature, Vol. 151, p. 183, 1943). In this paper you can read about

the intimate relationship between basic research and practical applications in this country, especially with regard to genetics and plant breeding.

According to Prof. G., MATHER has written that »the progress of genetics has not yet led to the marked advances in plant and animal breeding which has been so confidently expected in the past». Maybe the expectations were too high in the beginning of this century, but in our opinion remarkable results have anyhow been obtained in the relative short period of fifty years. This is discussed in detail by MÜNTZING (1950). Let us only remind you of such facts as the constancy of clones and pure lines owing to genotypical constancy; the fundamental difference between phenotype and genotype; the principle of genetic recombination; the discovery that quantitative characters may also be recombined, although they are generally conditioned by multiple factors which may be linked; the occurrence of genotypical adaptation to various habitats; the occurrence of spontaneous mutations and the possibility of inducing such mutations by external agents; the widespread occurrence of polyploidy and proof that polyploidy is involved in the origin of many of our most important cultivated plant species; the discovery that self-sterility is generally controlled by systems of multiple alleles which affect pollen germination and pollen tube growth; the demonstration that inbreeding in cross-fertilizing organisms leads to genetic differentiation and at the inbreeding minimum to homozygosity; and that intercrosses between inbred lines in certain combinations lead to products of great economical importance.

This list is far from complete, but in view of such facts it is an impenetrable mystery to us how you, Professor GLUSHCHENKO, or anybody else, can really state that genetics is a practically sterile science.

Our answer to you is already becoming too long but a few words must be said about inbreeding and outcrossing as a plant-breeding method. You have succeeded in finding some quotations (by HERIBERT NILSSON and FISHER) which to a certain extent support your attitude. Let us inform you, however, that one of us has discussed HERIBERT NILSSON's results in rye and finds that they do not seem to differ in any essential way from the evidence obtained in maize (MÜNTZING, 1945, pp. 156—158). You also ask why we do not use inbreeding and outcrossing in the breeding work with winter rye in Sweden. Let us first inform you that the breeding work with diploid rye is in other hands than our own, secondly, that experiments of this kind have already been carried out. MÜNTZING (1943) has reported results from single and double crosses between inbred lines in rye and obtained results of essentially the same type as those in maize. He also stressed the desirability of testing the double cross method in rye on a larger scale. That this has not yet been done is primarily owing to the fact that rye in contrast to maize is not monoecious but hermaphrodite, which prevents the easy mass emasculations and intercrosses which can be undertaken in maize. In principle, however, the two species react in the same way towards inbreeding (*cf.* also MÜNTZING, 1950, pp. 481—483).

Although the great field of research represented by the inbreeding and outbreeding phenomena will probably still give further results of interest in the future, we consider the main problems to be already solved. The theoretical

foundations were laid by such pioneers as SHULL, EAST and JONES; and from population studies, especially in *Drosophila* and in some plants, valuable data have more recently been obtained concerning the causes of inbreeding degeneration.

In your article you emphasize the remarkable biological results recently obtained in the Soviet Union, such as »the theory of phasic development of plants, the theory of vitality, the theory of the origin of cells from non-cellular living substance, *etc.* You also mention that you have yourself been working for a long time on the genesis of the plant cell.

This sounds, indeed, quite interesting, but we cannot accept such theories until they have been controlled and verified by other research workers outside the Soviet Union. To us some of these results at least seem a bit too good to be true. This includes the recent discoveries by LYSENKO (1951) concerning the sudden formation of new species. According to this author, single kernels of rye may be found in the ears of different wheat species, and from such kernels almost always typical rye plants grow up. A certain variety of *Triticum turgidum* was even more productive and gave rise not only to *Triticum durum* and *vulgare* but also to oats, 2-rowed and 4-rowed barley and to spring rye.

In LYSENKO's own words this is described as follows:

»It has been observed year after year when cultivating branched wheat (*Triticum turgidum*) on experimental plots of the Lenin Academy of Agricultural Sciences of the U.S.S.R and in a number of other localities that admixtures of soft and durum wheat, oats, 2- and 4-rowed barley and also spring rye appear in the crops.

All our observations led us to conclude that the original source of these admixtures was the branched wheat (*Triticum turgidum*) itself«.

Especially if the fields are not kept in good order this sudden species formation may have rather unpleasant consequences. Rudiments of weed species which are normally restricted to the interior of the cultivated plants get a chance to emerge and suddenly result in the formation of weed seeds in the ears of wheat or other cultivated plants. Already existing weed species may also suddenly generate new weed species by the same method. It is also suggested that similar processes may occur among animals and that such data will soon be discovered on the basis of the Michurin theory.

Especially as these theories are advanced by the leading Soviet biologist, Academician T. D. LYSENKO, we await, of course, with great interest the further thrilling results which may be expected in this new field of research. It is interesting to observe, however, that the priority belongs to a certain THOMAS JOHNSON, who in 1633 reported that he had found a grain of oats in an ear of wheat, and to OLE WORM, who in 1655 reported the finding of a grain of rye in an ear of barley (ZIRKLE, 1950, p. 48).

Although it would have been desirable to deal with several other aspects of Prof. GLUSHCHENKO's article we have to restrict ourselves to the main points discussed above. As emphasized already in our previous criticism, it is only by means of such discussions that we can ever hope to bridge the differences in opinion between the representatives of the Michurin biology and ourselves.

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